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(54) Title: ENVIRONMENTAL STRESS TOLERANCE GENES

(57) Abstract: Recombinant polynucleotides and methods for modifying the phenotype of a plant are provided. In particular, the phenotype that is being modified is a plant's environmental stress tolerance.

<b>ENVIRONMENTAL STRESS TOLERANCE GENES</b>
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**RELATED APPLICATION INFORMATION**

The present invention claims the benefit from US Provisional Patent Application Serial  
5 Nos. 60/166,228 filed November 17, 1999 and 60/197,899 filed April 17, 2000 and "Plant Trait  
Modification III" filed August 22, 2000.

**FIELD OF THE INVENTION**

This invention relates to the field of plant biology. More particularly, the present  
invention pertains to compositions and methods for phenotypically modifying a plant.

10

**BACKGROUND OF THE INVENTION**

Transcription factors can modulate gene expression, either increasing or  
decreasing (inducing or repressing) the rate of transcription. This modulation results in  
differential levels of gene expression at various developmental stages, in different tissues and cell  
types, and in response to different exogenous (e.g., environmental) and endogenous stimuli  
15 throughout the life cycle of the organism.

15

Because transcription factors are key controlling elements of biological  
pathways, altering the expression levels of one or more transcription factors can change entire  
biological pathways in an organism. For example, manipulation of the levels of selected  
transcription factors may result in increased expression of economically useful proteins or  
20 metabolic chemicals in plants or to improve other agriculturally relevant characteristics.  
Conversely, blocked or reduced expression of a transcription factor may reduce biosynthesis of  
unwanted compounds or remove an undesirable trait. Therefore, manipulating transcription  
factor levels in a plant offers tremendous potential in agricultural biotechnology for modifying a  
plant's traits.

25

The present invention provides novel transcription factors useful for modifying a  
plant's phenotype in desirable ways, such as modifying a plant's environmental stress tolerance.

**SUMMARY OF THE INVENTION**

In a first aspect, the invention relates to a recombinant polynucleotide comprising  
a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a  
30 polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a  
complementary nucleotide sequence thereof; (b) a nucleotide sequence encoding a polypeptide  
comprising a conservatively substituted variant of a polypeptide of (a); (c) a nucleotide sequence  
comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a

complementary nucleotide sequence thereof; (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c); (e) a nucleotide sequence which hybridizes under stringent conditions over substantially the entire length of a nucleotide sequence of one or more of: (a), (b), (c), or (d); (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e); (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide having a biological activity that modifies a plant's environmental stress tolerance; (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g); (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g); (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27. The recombinant polynucleotide may further comprise a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence. The invention also relates to compositions comprising at least two of the above described polynucleotides.

In a second aspect, the invention is an isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide described above.

In another aspect, the invention is a transgenic plant comprising one or more of the above described recombinant polynucleotides. In yet another aspect, the invention is a plant with altered expression levels of a polynucleotide described above or a plant with altered expression or activity levels of an above described polypeptide. Further, the invention may be a plant lacking a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27.

The plant may be a soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf, banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, or vegetable brassicas plant.

In a further aspect, the invention relates to a cloning or expression vector comprising the isolated or recombinant polynucleotide described above or cells comprising the cloning or expression vector.

In yet a further aspect, the invention relates to a composition produced by incubating a polynucleotide of the invention with a nuclease, a restriction enzyme, a polymerase; a polymerase and a primer; a cloning vector, or with a cell.

Furthermore, the invention relates to a method for producing a plant having  
5 improved environmental stress tolerance. The method comprises altering the expression of an isolated or recombinant polynucleotide of the invention or altering the expression or activity of a polypeptide of the invention in a plant to produce a modified plant, and selecting the modified plant for modified environmental stress tolerance.

In another aspect, the invention relates to a method of identifying a factor that is  
10 modulated by or interacts with a polypeptide encoded by a polynucleotide of the invention. The method comprises expressing a polypeptide encoded by the polynucleotide in a plant; and identifying at least one factor that is modulated by or interacts with the polypeptide. In one embodiment the method for identifying modulating or interacting factors is by detecting binding by the polypeptide to a promoter sequence, or by detecting interactions between an additional  
15 protein and the polypeptide in a yeast two hybrid system, or by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

In yet another aspect, the invention is a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest. The method comprises placing the molecule in contact with a plant comprising the polynucleotide or  
20 polypeptide encoded by the polynucleotide of the invention and monitoring one or more of the expression level of the polynucleotide in the plant, the expression level of the polypeptide in the plant, and modulation of an activity of the polypeptide in the plant.

In yet another aspect, the invention relates to an integrated system, computer or computer readable medium comprising one or more character strings corresponding to a  
25 polynucleotide of the invention, or to a polypeptide encoded by the polynucleotide. The integrated system, computer or computer readable medium may comprise a link between one or more sequence strings to a modified plant environmental stress tolerance phenotype.

In yet another aspect, the invention is a method for identifying a sequence similar or homologous to one or more polynucleotides of the invention, or one or more polypeptides  
30 encoded by the polynucleotides. The method comprises providing a sequence database; and, querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.



The method may further comprise of linking the one or more of the polynucleotides of the invention, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.

#### BRIEF DESCRIPTION OF THE DRAWINGS

5 Figure 1 provides a table of exemplary polynucleotide and polypeptide sequences of the invention. The table includes from left to right for each sequence: the SEQ ID No., the internal code reference number (GID), whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

10 Figure 2 provides a table of exemplary sequences that are homologous to other sequences provided in the Sequence Listing and that are derived from *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), identification of the homologous sequence, whether the sequence is a polynucleotide or polypeptide sequence, and identification of any conserved domains for the polypeptide sequences.

15 Figure 3 provides a table of exemplary sequences that are homologous to the sequences provided in Figures 1 and 2 and that are derived from plants other than *Arabidopsis thaliana*. The table includes from left to right: the SEQ ID No., the internal code reference number (GID), the unique GenBank sequence ID No. (NID), the probability that the comparison was generated by chance (P-value), and the species from which the homologous gene was identified.

20

#### DETAILED DESCRIPTION

The present invention relates to polynucleotides and polypeptides, e.g. for modifying phenotypes of plants.

25 In particular, the polynucleotides or polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance when the expression levels of the polynucleotides or expression levels or activity levels of the polypeptides are altered. Specifically, the polynucleotides and polypeptides are useful for modifying traits associated with a plant's environmental stress tolerance, such as freezing, chilling, heat, drought, water saturation, salt, photoconditions, radiation and ozone, or the like. Plants with altered expression of the  
30 polynucleotides or polypeptides of the invention are more tolerant to these environmental stresses compared with plants without altered expression levels.

The polynucleotides of the invention encode plant transcription factors. The plant transcription factors are derived, e.g., from *Arabidopsis thaliana* and can belong, e.g., to one

or more of the following transcription factor families: the AP2 (APETALA2) domain transcription factor family (Riechmann and Meyerowitz (1998) J. Biol. Chem. 379:633-646); the MYB transcription factor family (Martin and Paz-Ares (1997) Trends Genet. 13:67-73); the MADS domain transcription factor family (Riechmann and Meyerowitz (1997) J. Biol. Chem. 378:1079-1101); the WRKY protein family (Ishiguro and Nakamura (1994) Mol. Gen. Genet. 244:563-571); the ankyrin-repeat protein family (Zhang et al. (1992) Plant Cell 4:1575-1588); the miscellaneous protein (MISC) family (Kim et al. (1997) Plant J. 11:1237-1251); the zinc finger protein (Z) family (Klug and Schwabe (1995) FASEB J. 9: 597-604); the homeobox (HB) protein family (Duboule (1994) Guidebook to the Homeobox Genes, Oxford University Press); the CAAT-element binding proteins (Forsburg and Guarente (1989) Genes Dev. 3:1166-1178); the squamosa promoter binding proteins (SPB) (Klein et al. (1996) Mol. Gen. Genet. 196 250:7-16); the NAM protein family; the IAA/AUX proteins (Rouse et al. (1998) Science 279:1371-1373); the HLH/MYC protein family (Littlewood et al. (1994) Prot. Profile 1:639-709); the DNA-binding protein (DBP) family (Tucker et al. (1994) EMBO J. 13:2994-3002); the bZIP family of transcription factors (Foster et al. (1994) FASEB J. 8:192-200); the BPF-1 protein (Box P-binding factor) family (da Costa e Silva et al. (1993) Plant J. 4:125-135); and the golden protein (GLD) family (Hall et al. (1998) Plant Cell 10:925-936).

In addition to methods for modifying a plant phenotype by employing one or more polynucleotides and polypeptides of the invention described herein, the polynucleotides and polypeptides of the invention have a variety of additional uses. These uses include their use in the recombinant production (i.e, expression) of proteins; as regulators of plant gene expression, as diagnostic probes for the presence of complementary or partially complementary nucleic acids (including for detection of natural coding nucleic acids); as substrates for further reactions, e.g., mutation reactions, PCR reactions, or the like, of as substrates for cloning e.g., including digestion or ligation reactions, and for identifying exogenous or endogenous modulators of the transcription factors.

#### DEFINITIONS

A "polynucleotide" is a nucleic acid sequence comprising a plurality of polymerized nucleotide residues, e.g., at least about 15 consecutive polymerized nucleotide residues, optionally at least about 30 consecutive nucleotides, at least about 50 consecutive nucleotides. In many instances, a polynucleotide comprises a nucleotide sequence encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the polynucleotide may comprise a promoter, an intron, an enhancer region, a polyadenylation site, a translation initiation

site, 5' or 3' untranslated regions, a reporter gene, a selectable marker, or the like. The polynucleotide can be single stranded or double stranded DNA or RNA. The polynucleotide optionally comprises modified bases or a modified backbone. The polynucleotide can be, e.g., genomic DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned DNA,  
5 a synthetic DNA or RNA, or the like. The polynucleotide can comprise a sequence in either sense or antisense orientations.

A "recombinant polynucleotide" is a polynucleotide that is not in its native state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or the polynucleotide is in a context other than that in which it is naturally found, e.g., separated from  
10 nucleotide sequences with which it typically is in proximity in nature, or adjacent (or contiguous with) nucleotide sequences with which it typically is not in proximity. For example, the sequence at issue can be cloned into a vector, or otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide whether naturally occurring or recombinant, that is present outside the cell in which it is typically found in nature, whether  
15 purified or not. Optionally, an isolated polynucleotide is subject to one or more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation, precipitation, or the like.

A "recombinant polypeptide" is a polypeptide produced by translation of a recombinant polynucleotide. An "isolated polypeptide," whether a naturally occurring or a  
20 recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in its natural state in a wild type cell, e.g., more than about 5% enriched, more than about 10% enriched, or more than about 20%, or more than about 50%, or more, enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched relative to wild type standardized at 100%. Such an enrichment is not the result of a natural response of a wild type plant. Alternatively, or  
25 additionally, the isolated polypeptide is separated from other cellular components with which it is typically associated, e.g., by any of the various protein purification methods herein.

The term "transgenic plant" refers to a plant that contains genetic material, not found in a wild type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA  
30 insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under

regulatory control of) to appropriate inducible or constitutive regulatory sequences that allow for the expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to *in vitro* systems that mimic biochemical or cellular components or processes in a cell.

The phrase "ectopically expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild type plant or a reference plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild type plant, or by expression at a time other than at the time the sequence is expressed in the wild type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "fragment" or "domain," with respect to a polypeptide, refers to a subsequence of the polypeptide. In some cases, the fragment or domain, is a subsequence of the polypeptide which performs at least one biological function of the intact polypeptide in substantially the same manner, or to a similar extent, as does the intact polypeptide. For example, a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA binding domain that binds to a DNA promoter region, an activation domain or a domain for protein-protein interactions. Fragments can vary in size from as few as 6 amino acids to the full length of the intact polypeptide, but are preferably at least about 30 amino acids in length and more preferably at least about 60 amino acids in length. In reference to a nucleotide sequence, "a fragment" refers to any subsequence of a polynucleotide, typically, of at least consecutive about 15 nucleotides, preferably at least about 30 nucleotides, more preferably at least about 50, of any of the sequences provided herein.

The term "trait" refers to a physiological, morphological, biochemical or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic

is visible to the human eye, such as seed or plant size, or can be measured by available biochemical techniques, such as the protein, starch or oil content of seed or leaves or by the observation of the expression level of genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays or reporter gene expression systems, or by agricultural observations such as stress tolerance, yield or pathogen tolerance.

"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease in an observed trait (difference), at least a 5% difference, at least about a 10% difference, at least about a 20% difference, at least about a 30%, at least about a 50%, at least about a 70%, or at least about a 100%, or an even greater difference. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution of the trait in the plants compared with the distribution observed in wild type plant.

Trait modifications of particular interest include those to seed (such as embryo or endosperm), fruit, root, flower, leaf, stem, shoot, seedling or the like, including: enhanced tolerance to environmental conditions including freezing, chilling, heat, drought, water saturation, radiation and ozone; improved tolerance to microbial, fungal or viral diseases; improved tolerance to pest infestations, including nematodes, mollicutes, parasitic higher plants or the like; decreased herbicide sensitivity; improved tolerance of heavy metals or enhanced ability to take up heavy metals; improved growth under poor photoconditions (e.g., low light and/or short day length), or changes in expression levels of genes of interest. Other phenotype that can be modified relate to the production of plant metabolites, such as variations in the production of taxol, tocopherol, tocotrienol, sterols, phytosterols, vitamins, wax monomers, anti-oxidants, amino acids, lignins, cellulose, tannins, prenolipids (such as chlorophylls and carotenoids), glucosinolates, and terpenoids, enhanced or compositionally altered protein or oil production (especially in seeds), or modified sugar (insoluble or soluble) and/or starch composition. Physical plant characteristics that can be modified include cell development (such as the number of trichomes), fruit and seed size and number, yields of plant parts such as stems, leaves and roots, the stability of the seeds during storage, characteristics of the seed pod (e.g., susceptibility to shattering), root hair length and quantity, internode distances, or the quality of seed coat. Plant growth characteristics that can be modified include growth rate, germination rate of seeds, vigor of plants and seedlings, leaf and flower senescence, male sterility, apomixis, flowering time,

flower abscission, rate of nitrogen uptake, biomass or transpiration characteristics, as well as plant architecture characteristics such as apical dominance, branching patterns, number of organs, organ identity, organ shape or size.

#### POLYPEPTIDES AND POLYNUCLEOTIDES OF THE INVENTION

5                   The present invention provides, among other things, transcription factors (TFs), and transcription factor homologue polypeptides, and isolated or recombinant polynucleotides encoding the polypeptides. These polypeptides and polynucleotides may be employed to modify a plant's environmental stress tolerance.

10                   Exemplary polynucleotides encoding the polypeptides of the invention were identified in the *Arabidopsis thaliana* GenBank database using publicly available sequence analysis programs and parameters. Sequences initially identified were then further characterized to identify sequences comprising specified sequence strings corresponding to sequence motifs present in families of known transcription factors. Polynucleotide sequences meeting such criteria were confirmed as transcription factors.

15                   Additional polynucleotides of the invention were identified by screening *Arabidopsis thaliana* and/or other plant cDNA libraries with probes corresponding to known transcription factors under low stringency hybridization conditions. Additional sequences, including full length coding sequences were subsequently recovered by the rapid amplification of cDNA ends (RACE) procedure, using a commercially available kit according to the  
20                   manufacturer's instructions. Where necessary, multiple rounds of RACE are performed to isolate 5' and 3' ends. The full length cDNA was then recovered by a routine end-to-end polymerase chain reaction (PCR) using primers specific to the isolated 5' and 3' ends. Exemplary sequences are provided in the Sequence Listing.

25                   The polynucleotides of the invention were ectopically expressed in overexpressor or knockout plants and changes in the environmental stress tolerance of the plants was observed. Therefore, the polynucleotides and polypeptides can be employed to improve the environmental stress resistance of plants.

#### Making polynucleotides

30                   The polynucleotides of the invention include sequences that encode transcription factors and transcription factor homologue polypeptides and sequences complementary thereto, as well as unique fragments of coding sequence, or sequence complementary thereto. Such polynucleotides can be, e.g., DNA or RNA, e.g., mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, oligonucleotides, etc. The polynucleotides are either double-stranded or

single-stranded, and include either, or both sense (i.e., coding) sequences and antisense (i.e., non-coding, complementary) sequences. The polynucleotides include the coding sequence of a transcription factor, or transcription factor homologue polypeptide, in isolation, in combination with additional coding sequences (e.g., a purification tag, a localization signal, as a fusion-protein, as a pre-protein, or the like), in combination with non-coding sequences (e.g., introns or  
5 introns, regulatory elements such as promoters, enhancers, terminators, and the like), and/or in a vector or host environment in which the polynucleotide encoding a transcription factor or transcription factor homologue polypeptide is an endogenous or exogenous gene.

A variety of methods exist for producing the polynucleotides of the invention.  
10 Procedures for identifying and isolating DNA clones are well known to those of skill in the art, and are described in, e.g., Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA ("Berger"); Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989 ("Sambrook") and Current Protocols in Molecular Biology,  
15 F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2000) ("Ausubel").

Alternatively, polynucleotides of the invention, can be produced by a variety of in vitro amplification methods adapted to the present invention by appropriate selection of specific or degenerate primers. Examples of protocols sufficient to direct persons of skill through  
20 in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qbeta-replicase amplification and other RNA polymerase mediated techniques (e.g., NASBA), e.g., for the production of the homologous nucleic acids of the invention are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., (1987) PCR Protocols A Guide to Methods and Applications (Innis et al. eds) Academic Press Inc. San Diego, CA (1990) (Innis).  
25 Improved methods for cloning in vitro amplified nucleic acids are described in Wallace et al., U.S. Pat. No. 5,426,039. Improved methods for amplifying large nucleic acids by PCR are summarized in Cheng et al. (1994) Nature 369: 684-685 and the references cited therein, in which PCR amplicons of up to 40kb are generated. One of skill will appreciate that essentially any RNA can be converted into a double stranded DNA suitable for restriction digestion, PCR  
30 expansion and sequencing using reverse transcriptase and a polymerase. See, e.g., Ausubel, Sambrook and Berger, *all supra*.

Alternatively, polynucleotides and oligonucleotides of the invention can be assembled from fragments produced by solid-phase synthesis methods. Typically, fragments of up to approximately 100 bases are individually synthesized and then enzymatically or chemically

ligated to produce a desired sequence, e.g., a polynucleotide encoding all or part of a transcription factor. For example, chemical synthesis using the phosphoramidite method is described, e.g., by Beaucage et al. (1981) Tetrahedron Letters 22:1859-69; and Matthes et al. (1984) EMBO J. 3:801-5. According to such methods, oligonucleotides are synthesized, purified, annealed to their complementary strand, ligated and then optionally cloned into suitable vectors. And if so desired, the polynucleotides and polypeptides of the invention can be custom ordered from any of a number of commercial suppliers.

#### HOMOLOGOUS SEQUENCES

Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided in the Sequence Listing, derived from *Arabidopsis thaliana* or from other plants of choice are also an aspect of the invention. Homologous sequences can be derived from any plant including monocots and dicots and in particular agriculturally important plant species, including but not limited to, crops such as soybean, wheat, corn, potato, cotton, rice, oilseed rape (including canola), sunflower, alfalfa, sugarcane and turf; or fruits and vegetables, such as banana, blackberry, blueberry, strawberry, and raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits (such as apple, peach, pear, cherry and plum) and vegetable brassicas (such as broccoli, cabbage, cauliflower, brussel sprouts and kohlrabi). Other crops, fruits and vegetables whose phenotype can be changed include barley, rye, millet, sorghum, currant, avocado, citrus fruits such as oranges, lemons, grapefruit and tangerines, artichoke, cherries, nuts such as the walnut and peanut, endive, leek, roots, such as arrowroot, beet, cassava, turnip, radish, yam, and sweet potato, and beans. The homologous sequences may also be derived from woody species, such as pine, poplar and eucalyptus.

Transcription factors that are homologous to the listed sequences will typically share at least about 30% amino acid sequence identity. More closely related transcription factors can share at least about 50%, about 60%, about 65%, about 70%, about 75% or about 80% or about 90% or about 95% or about 98% or more sequence identity with the listed sequences. Factors that are most closely related to the listed sequences share, e.g., at least about 85%, about 90% or about 95% or more % sequence identity to the listed sequences. At the nucleotide level, the sequences will typically share at least about 40% nucleotide sequence identity, preferably at least about 50%, about 60%, about 70% or about 80% sequence identity, and more preferably about 85%, about 90%, about 95% or about 97% or more sequence identity to one or more of the



listed sequences. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein. Conserved domains within a transcription factor family may exhibit a higher degree of sequence homology, such as at least 65% sequence identity including conservative substitutions, and preferably at least 80% sequence identity.

#### Identifying Nucleic Acids by Hybridization

Polynucleotides homologous to the sequences illustrated in the Sequence Listing can be identified, e.g., by hybridization to each other under stringent or under highly stringent conditions. Single stranded polynucleotides hybridize when they associate based on a variety of well characterized physico-chemical forces, such as hydrogen bonding, solvent exclusion, base stacking and the like. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number), as described in more detail in the references cited above.

An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions will typically hybridize to a probe based on either the entire cDNA or selected portions, e.g., to a unique subsequence, of the cDNA under wash conditions of 0.2x SSC to 2.0 x SSC, 0.1% SDS at 50-65° C, for example 0.2 x SSC, 0.1% SDS at 65° C. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50° C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

As another example, stringent conditions can be selected such that an oligonucleotide that is perfectly complementary to the coding oligonucleotide hybridizes to the coding oligonucleotide with at least about a 5-10x higher signal to noise ratio than the ratio for hybridization of the perfectly complementary oligonucleotide to a nucleic acid encoding a transcription factor known as of the filing date of the application. Conditions can be selected such that a higher signal to noise ratio is observed in the particular assay which is used, e.g., about 15x, 25x, 35x, 50x or more. Accordingly, the subject nucleic acid hybridizes to the unique

coding oligonucleotide with at least a 2x higher signal to noise ratio as compared to hybridization of the coding oligonucleotide to a nucleic acid encoding known polypeptide. Again, higher signal to noise ratios can be selected, e.g., about 5x, 10x, 25x, 35x, 50x or more. The particular signal will depend on the label used in the relevant assay, e.g., a fluorescent label, a colorimetric label, a radio active label, or the like.

Alternatively, transcription factor homologue polypeptides can be obtained by screening an expression library using antibodies specific for one or more transcription factors. With the provision herein of the disclosed transcription factor, and transcription factor homologue nucleic acid sequences, the encoded polypeptide(s) can be expressed and purified in a heterologous expression system (e.g., *E. coli*) and used to raise antibodies (monoclonal or polyclonal) specific for the polypeptide(s) in question. Antibodies can also be raised against synthetic peptides derived from transcription factor, or transcription factor homologue, amino acid sequences. Methods of raising antibodies are well known in the art and are described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Such antibodies can then be used to screen an expression library produced from the plant from which it is desired to clone additional transcription factor homologues, using the methods described above. The selected cDNAs can be confirmed by sequencing and enzymatic activity.

#### SEQUENCE VARIATIONS

It will readily be appreciated by those of skill in the art, that any of a variety of polynucleotide sequences are capable of encoding the transcription factors and transcription factor homologue polypeptides of the invention. Due to the degeneracy of the genetic code, many different polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

For example, Table 1 illustrates, e.g., that the codons AGC, AGT, TCA, TCC, TCG, and TCT all encode the same amino acid: serine. Accordingly, at each position in the sequence where there is a codon encoding serine, any of the above trinucleotide sequences can be used without altering the encoded polypeptide.

**Table 1**

Amino acids			Codon							
Alanine	Ala	A	GCA	GCC	GCG	GCU				
Cysteine	Cys	C	TGC	TGT						
Aspartic acid	Asp	D	GAC	GAT						
Glutamic acid	Glu	E	GAA	GAG						
Phenylalanine	Phe	F	TTC	TTT						
Glycine	Gly	G	GGA	GGC	GGG	GGT				
Histidine	His	H	CAC	CAT						
Isoleucine	Ile	I	ATA	ATC	ATT					
Lysine	Lys	K	AAA	AAG						
Leucine	Leu	L	TTA	TTG	CTA	CTC	CTG	CTT		
Methionine	Met	M	ATG							
Asparagine	Asn	N	AAC	AAT						
Proline	Pro	P	CCA	CCC	CCG	CCT				
Glutamine	Gln	Q	CAA	CAG						
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGT		
Serine	Ser	S	AGC	AGT	TCA	TCC	TCG	TCT		
Threonine	Thr	T	ACA	ACC	ACG	ACT				
Valine	Val	V	GTA	GTC	GTG	GTT				
Tryptophan	Trp	W	TGG							
Tyrosine	Tyr	Y	TAC	TAT						

Sequence alterations that do not change the amino acid sequence encoded by the polynucleotide are termed "silent" variations. With the exception of the codons ATG and TGG, encoding methionine and tryptophan, respectively, any of the possible codons for the same amino acid can be substituted by a variety of techniques, e.g., site-directed mutagenesis, available in the art. Accordingly, any and all such variations of a sequence selected from the above table are a feature of the invention.

In addition to silent variations, other conservative variations that alter one, or a few amino acids in the encoded polypeptide, can be made without altering the function of the polypeptide, these conservative variants are, likewise, a feature of the invention.

For example, substitutions, deletions and insertions introduced into the sequences provided in the Sequence Listing are also envisioned by the invention. Such sequence modifications can be engineered into a sequence by site-directed mutagenesis (Wu (ed.) Meth. Enzymol. (1993) vol. 217, Academic Press) or the other methods noted below. Amino acid substitutions are typically of single residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. In preferred embodiments, deletions or insertions are made in adjacent pairs, e.g., a deletion of two residues or insertion of two residues. Substitutions, deletions, insertions or any combination thereof can be

combined to arrive at a sequence. The mutations that are made in the polynucleotide encoding the transcription factor should not place the sequence out of reading frame and should not create complementary regions that could produce secondary mRNA structure. Preferably, the polypeptide encoded by the DNA performs the desired function.

- 5                   Conservative substitutions are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the Table 2 when it is desired to maintain the activity of the protein. Table 2 shows amino acids which can be substituted for an amino acid in a protein and which are typically regarded as conservative substitutions.

10

**Table 2**

<b>Residue</b>	<b>Conservative Substitutions</b>
Ala	Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Gln	Asn
Cys	Ser
Glu	Asp
Gly	Pro
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr; Gly
Thr	Ser; Val
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Substitutions that are less conservative than those in Table 2 can be selected by picking residues that differ more significantly in their effect on maintaining (a) the structure of

the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

10 FURTHER MODIFYING SEQUENCES OF THE INVENTION—MUTATION/ FORCED EVOLUTION

In addition to generating silent or conservative substitutions as noted, above, the present invention optionally includes methods of modifying the sequences of the Sequence Listing. In the methods, nucleic acid or protein modification methods are used to alter the given sequences to produce new sequences and/or to chemically or enzymatically modify given sequences to change the properties of the nucleic acids or proteins.

Thus, in one embodiment, given nucleic acid sequences are modified, e.g., according to standard mutagenesis or artificial evolution methods to produce modified sequences. For example, Ausubel, *supra*, provides additional details on mutagenesis methods. Artificial forced evolution methods are described, e.g., by Stemmer (1994) *Nature* 370:389-391, and Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751. Many other mutation and evolution methods are also available and expected to be within the skill of the practitioner.

Similarly, chemical or enzymatic alteration of expressed nucleic acids and polypeptides can be performed by standard methods. For example, sequence can be modified by addition of lipids, sugars, peptides, organic or inorganic compounds, by the inclusion of modified nucleotides or amino acids, or the like. For example, protein modification techniques are illustrated in Ausubel, *supra*. Further details on chemical and enzymatic modifications can be found herein. These modification methods can be used to modify any given sequence, or to modify any sequence produced by the various mutation and artificial evolution modification methods noted herein.

Accordingly, the invention provides for modification of any given nucleic acid by mutation, evolution, chemical or enzymatic modification, or other available methods, as well as for the products produced by practicing such methods, e.g., using the sequences herein as a starting substrate for the various modification approaches.

For example, optimized coding sequence containing codons preferred by a particular prokaryotic or eukaryotic host can be used e.g., to increase the rate of translation or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, as compared with transcripts produced using a non-optimized sequence. Translation stop codons can also be modified to reflect host preference. For example, preferred stop codons for *S. cerevisiae* and mammals are TAA and TGA, respectively. The preferred stop codon for monocotyledonous plants is TGA, whereas insects and *E. coli* prefer to use TAA as the stop codon.

The polynucleotide sequences of the present invention can also be engineered in order to alter a coding sequence for a variety of reasons, including but not limited to, alterations which modify the sequence to facilitate cloning, processing and/or expression of the gene product. For example, alterations are optionally introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to introduce splice sites, etc.

Furthermore, a fragment or domain derived from any of the polypeptides of the invention can be combined with domains derived from other transcription factors or synthetic domains to modify the biological activity of a transcription factor. For instance, a DNA binding domain derived from a transcription factor of the invention can be combined with the activation domain of another transcription factor or with a synthetic activation domain. A transcription activation domain assists in initiating transcription from a DNA binding site. Examples include the transcription activation region of VP16 or GAL4 (Moore et al. (1998) Proc. Natl. Acad. Sci. USA 95: 376-381; and Aoyama et al. (1995) Plant Cell 7:1773-1785), peptides derived from bacterial sequences (Ma and Ptashne (1987) Cell 51: 113-119) and synthetic peptides (Giniger and Ptashne, (1987) Nature 330:670-672).

## EXPRESSION AND MODIFICATION OF POLYPEPTIDES

Typically, polynucleotide sequences of the invention are incorporated into recombinant DNA (or RNA) molecules that direct expression of polypeptides of the invention in appropriate host cells, transgenic plants, in vitro translation systems, or the like. Due to the inherent degeneracy of the genetic code, nucleic acid sequences which encode substantially the same or a functionally equivalent amino acid sequence can be substituted for any listed sequence to provide for cloning and expressing the relevant homologue.

### Vectors, Promoters and Expression Systems

The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC),  
5 a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

10 General texts which describe molecular biological techniques useful herein, including the use and production of vectors, promoters and many other relevant topics, include Berger, Sambrook and Ausubel, *supra*. Any of the identified sequences can be incorporated into a cassette or vector, e.g., for expression in plants. A number of expression vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described  
15 including those described in Weissbach and Weissbach, (1989) Methods for Plant Molecular Biology, Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technology 3: 637-642,  
20 for dicotyledonous plants.

Alternatively, non-Ti vectors can be used to transfer the DNA into monocotyledonous plants and cells by using free DNA delivery techniques. Such methods can involve, for example, the use of liposomes, electroporation, microprojectile bombardment, silicon carbide whiskers, and viruses. By using these methods transgenic plants such as wheat, rice  
25 (Christou (1991) Bio/Technology 9: 957-962) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technology 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48, and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) Nature Biotech  
30 14: 745-750).

Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant transformation vectors typically also contain a promoter (e.g., a regulatory region controlling inducible or constitutive, environmentally-or

developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

Examples of constitutive plant promoters which can be useful for expressing the  
 5 TF sequence include: the cauliflower mosaic virus (CaMV) 35S promoter, which confers constitutive, high-level expression in most plant tissues (*see, e.g., Odel et al. (1985) Nature 313:810*); the nopaline synthase promoter (An et al. (1988) Plant Physiol 88:547); and the octopine synthase promoter (Fromm et al. (1989) Plant Cell 1: 977).

A variety of plant gene promoters that regulate gene expression in response to  
 10 environmental, hormonal, chemical, developmental signals, and in a tissue-active manner can be used for expression of a TF sequence in plants. Choice of a promoter is based largely on the phenotype of interest and is determined by such factors as tissue (e.g., seed, fruit, root, pollen, vascular tissue, flower, carpel, etc.), inducibility (e.g., in response to wounding, heat, cold, drought, light, pathogens, etc.), timing, developmental stage, and the like. Numerous known  
 15 promoters have been characterized and can favorably be employed to promote expression of a polynucleotide of the invention in a transgenic plant or cell of interest. For example, tissue specific promoters include: seed-specific promoters (such as the napin, phaseolin or DC3 promoter described in US Pat. No. 5,773,697), fruit-specific promoters that are active during fruit ripening (such as the *dru 1* promoter (US Pat. No. 5,783,393), or the 2A11 promoter (US Pat. No.  
 20 4,943,674) and the tomato polygalacturonase promoter (Bird et al. (1988) Plant Mol Biol 11:651), root-specific promoters, such as those disclosed in US Patent Nos. 5,618,988, 5,837,848 and 5,905,186, pollen-active promoters such as PTA29, PTA26 and PTA13 (US Pat. No. 5,792,929), promoters active in vascular tissue (Ringli and Keller (1998) Plant Mol Biol 37:977-988), flower-specific (Kaiser et al. (1995) Plant Mol Biol 28:231-243), pollen (Baerson et al. (1994) Plant Mol Biol 26:1947-1959), carpels (Ohl et al. (1990) Plant Cell 2:837-848), pollen and ovules (Baerson  
 25 et al. (1993) Plant Mol Biol 22:255-267), auxin-inducible promoters (such as that described in van der Kop et al. (1999) Plant Mol Biol 39:979-990 or Baumann et al. (1999) Plant Cell 11:323-334), cytokinin-inducible promoter (Guevara-Garcia (1998) Plant Mol Biol 38:743-753), promoters responsive to gibberellin (Shi et al. (1998) Plant Mol Biol 38:1053-1060, Willmott et  
 30 al. (1998) 38:817-825) and the like. Additional promoters are those that elicit expression in response to heat (Ainley et al. (1993) Plant Mol Biol 22: 13-23), light (e.g., the pea *rbcS-3A* promoter, Kuhlemeier et al. (1989) Plant Cell 1:471, and the maize *rbcS* promoter, Schaffner and Sheen (1991) Plant Cell 3: 997); wounding (e.g., *wun1*, Siebertz et al. (1989) Plant Cell 1: 961); pathogens (such as the PR-1 promoter described in Buchel et al. (1999) Plant Mol. Biol. 40:387-



396, and the PDF1.2 promoter described in Manners et al. (1998) Plant Mol. Biol. 38:1071-80), and chemicals such as methyl jasmonate or salicylic acid (Gatz et al. (1997) Plant Mol Biol 48: 89-108). In addition, the timing of the expression can be controlled by using promoters such as those acting at senescence (An and Amazon (1995) Science 270: 1986-1988); or late seed development  
5 (Odell et al. (1994) Plant Physiol 106:447-458).

Plant expression vectors can also include RNA processing signals that can be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors can include additional regulatory sequences from the 3'-untranslated region of plant genes, e.g., a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II  
10 terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

#### Additional Expression Elements

Specific initiation signals can aid in efficient translation of coding sequences. These signals can include, e.g., the ATG initiation codon and adjacent sequences. In cases where a coding sequence, its initiation codon and upstream sequences are inserted into the appropriate  
15 expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence (e.g., a mature protein coding sequence), or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon can be separately provided. The initiation codon is provided in the correct reading frame to facilitate transcription. Exogenous transcriptional elements and initiation codons can be of various origins,  
20 both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers appropriate to the cell system in use.

#### Expression Hosts

The present invention also relates to host cells which are transduced with vectors of the invention, and the production of polypeptides of the invention (including fragments  
25 thereof) by recombinant techniques. Host cells are genetically engineered (i.e, nucleic acids are introduced, e.g., transduced, transformed or transfected) with the vectors of this invention, which may be, for example, a cloning vector or an expression vector comprising the relevant nucleic acids herein. The vector is optionally a plasmid, a viral particle, a phage, a naked nucleic acids, *etc.* The engineered host cells can be cultured in conventional nutrient media modified as  
30 appropriate for activating promoters, selecting transformants, or amplifying the relevant gene. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to those skilled in the art and in the references cited herein, including, Sambrook and Ausubel.

The host cell can be a eukaryotic cell, such as a yeast cell, or a plant cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Plant protoplasts are also suitable for some applications. For example, the DNA fragments are introduced into plant tissues, cultured plant cells or plant protoplasts by standard methods including electroporation (Fromm et al., (1985) Proc. Natl. Acad. Sci. USA 82, 5824, infection by viral vectors such as cauliflower mosaic virus (CaMV) (Hohn et al., (1982) Molecular Biology of Plant Tumors, (Academic Press, New York) pp. 549-560; US 4,407,956), high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface (Klein et al., (1987) Nature 327, 70-73), use of pollen as vector (WO 85/01856), or use of *Agrobacterium tumefaciens* or *A. rhizogenes* carrying a T-DNA plasmid in which DNA fragments are cloned. The T-DNA plasmid is transmitted to plant cells upon infection by *Agrobacterium tumefaciens*, and a portion is stably integrated into the plant genome (Horsch et al. (1984) Science 233:496-498; Fraley et al. (1983) Proc. Natl. Acad. Sci. USA 80, 4803).

The cell can include a nucleic acid of the invention which encodes a polypeptide, wherein the cells expresses a polypeptide of the invention. The cell can also include vector sequences, or the like. Furthermore, cells and transgenic plants which include any polypeptide or nucleic acid above or throughout this specification, e.g., produced by transduction of a vector of the invention, are an additional feature of the invention.

For long-term, high-yield production of recombinant proteins, stable expression can be used. Host cells transformed with a nucleotide sequence encoding a polypeptide of the invention are optionally cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein or fragment thereof produced by a recombinant cell may be secreted, membrane-bound, or contained intracellularly, depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides encoding mature proteins of the invention can be designed with signal sequences which direct secretion of the mature polypeptides through a prokaryotic or eukaryotic cell membrane.

#### Modified Amino Acids

Polypeptides of the invention may contain one or more modified amino acids.

The presence of modified amino acids may be advantageous in, for example, increasing polypeptide half-life, reducing polypeptide antigenicity or toxicity, increasing polypeptide storage stability, or the like. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production or modified by synthetic or chemical means.

Non-limiting examples of a modified amino acid include incorporation or other use of acetylated amino acids, glycosylated amino acids, sulfated amino acids, prenylated (e.g., farnesylated, geranylgeranylated) amino acids, PEG modified (e.g., "PEGylated") amino acids, biotinylated amino acids, carboxylated amino acids, phosphorylated amino acids, etc. References  
5 adequate to guide one of skill in the modification of amino acids are replete throughout the literature.

#### IDENTIFICATION OF ADDITIONAL FACTORS

A transcription factor provided by the present invention can also be used to identify additional endogenous or exogenous molecules that can affect a phenotype or trait of  
10 interest. On the one hand, such molecules include organic (small or large molecules) and/or inorganic compounds that affect expression of (i.e., regulate) a particular transcription factor. Alternatively, such molecules include endogenous molecules that are acted upon either at a transcriptional level by a transcription factor of the invention to modify a phenotype as desired. For example, the transcription factors can be employed to identify one or more downstream gene  
15 with which is subject to a regulatory effect of the transcription factor. In one approach, a transcription factor or transcription factor homologue of the invention is expressed in a host cell, e.g., a transgenic plant cell, tissue or explant, and expression products, either RNA or protein, of likely or random targets are monitored, e.g., by hybridization to a microarray of nucleic acid probes corresponding to genes expressed in a tissue or cell type of interest, by two-dimensional  
20 gel electrophoresis of protein products, or by any other method known in the art for assessing expression of gene products at the level of RNA or protein. Alternatively, a transcription factor of the invention can be used to identify promoter sequences (i.e., binding sites) involved in the regulation of a downstream target. After identifying a promoter sequence, interactions between the transcription factor and the promoter sequence can be modified by changing specific  
25 nucleotides in the promoter sequence or specific amino acids in the transcription factor that interact with the promoter sequence to alter a plant trait. Typically, transcription factor DNA binding sites are identified by gel shift assays. After identifying the promoter regions, the promoter region sequences can be employed in double-stranded DNA arrays to identify molecules that affect the interactions of the transcription factors with their promoters (Bulyk et al.  
30 (1999) Nature Biotechnology 17:573-577).

The identified transcription factors are also useful to identify proteins that modify the activity of the transcription factor. Such modification can occur by covalent modification, such as by phosphorylation, or by protein-protein (homo or-heteropolymer) interactions. Any

method suitable for detecting protein-protein interactions can be employed. Among the methods that can be employed are co-immunoprecipitation, cross-linking and co-purification through gradients or chromatographic columns, and the two-hybrid yeast system.

The two-hybrid system detects protein interactions in vivo and is described in Chien, et al., (1991), Proc. Natl. Acad. Sci. USA 88, 9578-9582 and is commercially available from Clontech (Palo Alto, Calif.). In such a system, plasmids are constructed that encode two hybrid proteins: one consists of the DNA-binding domain of a transcription activator protein fused to the TF polypeptide and the other consists of the transcription activator protein's activation domain fused to an unknown protein that is encoded by a cDNA that has been recombined into the plasmid as part of a cDNA library. The DNA-binding domain fusion plasmid and the cDNA library are transformed into a strain of the yeast *Saccharomyces cerevisiae* that contains a reporter gene (e.g., lacZ) whose regulatory region contains the transcription activator's binding site. Either hybrid protein alone cannot activate transcription of the reporter gene. Interaction of the two hybrid proteins reconstitutes the functional activator protein and results in expression of the reporter gene, which is detected by an assay for the reporter gene product. Then, the library plasmids responsible for reporter gene expression are isolated and sequenced to identify the proteins encoded by the library plasmids. After identifying proteins that interact with the transcription factors, assays for compounds that interfere with the TF protein-protein interactions can be preformed.

## 20 IDENTIFICATION OF MODULATORS

In addition to the intracellular molecules described above, extracellular molecules that alter activity or expression of a transcription factor, either directly or indirectly, can be identified. For example, the methods can entail first placing a candidate molecule in contact with a plant or plant cell. The molecule can be introduced by topical administration, such as spraying or soaking of a plant, and then the molecule's effect on the expression or activity of the TF polypeptide or the expression of the polynucleotide monitored. Changes in the expression of the TF polypeptide can be monitored by use of polyclonal or monoclonal antibodies, gel electrophoresis or the like. Changes in the expression of the corresponding polynucleotide sequence can be detected by use of microarrays, Northern blots, quantitative PCR, or any other technique for monitoring changes in mRNA expression. These techniques are exemplified in Ausubel et al. (eds) Current Protocols in Molecular Biology, John Wiley & Sons (1998). Such changes in the expression levels can be correlated with modified plant traits and thus identified

molecules can be useful for soaking or spraying on fruit, vegetable and grain crops to modify traits in plants.

Essentially any available composition can be tested for modulatory activity of expression or activity of any nucleic acid or polypeptide herein. Thus, available libraries of compounds such as chemicals, polypeptides, nucleic acids and the like can be tested for modulatory activity. Often, potential modulator compounds can be dissolved in aqueous or organic (e.g., DMSO-based) solutions for easy delivery to the cell or plant of interest in which the activity of the modulator is to be tested. Optionally, the assays are designed to screen large modulator composition libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

In one embodiment, high throughput screening methods involve providing a combinatorial library containing a large number of potential compounds (potential modulator compounds). Such "combinatorial chemical libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as target compounds.

A combinatorial chemical library can be, e.g., a collection of diverse chemical compounds generated by chemical synthesis or biological synthesis. For example, a combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (e.g., in one example, amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound of a set length). Exemplary libraries include peptide libraries, nucleic acid libraries, antibody libraries (see, e.g., Vaughn et al. (1996) *Nature Biotechnology*, 14(3):309-314 and PCT/US96/10287), carbohydrate libraries (see, e.g., Liang et al. *Science* (1996) 274:1520-1522 and U.S. Patent 5,593,853), peptide nucleic acid libraries (see, e.g., U.S. Patent 5,539,083), and small organic molecule libraries (see, e.g., benzodiazepines, Baum *C&EN* Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent 5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337) and the like.

Preparation and screening of combinatorial or other libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (see, e.g., U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton et al. *Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used.

In addition, as noted, compound screening equipment for high-throughput screening is generally available, e.g., using any of a number of well known robotic systems that have also been developed for solution phase chemistries useful in assay systems. These systems include automated workstations including an automated synthesis apparatus and robotic systems  
5 utilizing robotic arms. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput screening of potential modulators. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Indeed, entire high throughput screening systems are commercially available.  
10 These systems typically automate entire procedures including all sample and reagent pipetting, liquid dispensing, timed incubations, and final readings of the microplate in detector(s) appropriate for the assay. These configurable systems provide high throughput and rapid start up as well as a high degree of flexibility and customization. Similarly, microfluidic implementations of screening are also commercially available.

15 The manufacturers of such systems provide detailed protocols the various high throughput. Thus, for example, Zymark Corp. provides technical bulletins describing screening systems for detecting the modulation of gene transcription, ligand binding, and the like. The integrated systems herein, in addition to providing for sequence alignment and, optionally, synthesis of relevant nucleic acids, can include such screening apparatus to identify modulators  
20 that have an effect on one or more polynucleotides or polypeptides according to the present invention.

In some assays it is desirable to have positive controls to ensure that the components of the assays are working properly. At least two types of positive controls are appropriate. That is, known transcriptional activators or inhibitors can be incubated with  
25 cells/plants/ etc. in one sample of the assay, and the resulting increase/decrease in transcription can be detected by measuring the resulting increase in RNA/ protein expression, etc., according to the methods herein. It will be appreciated that modulators can also be combined with transcriptional activators or inhibitors to find modulators which inhibit transcriptional activation or transcriptional repression. Either expression of the nucleic acids and proteins herein or any  
30 additional nucleic acids or proteins activated by the nucleic acids or proteins herein, or both, can be monitored.

In an embodiment, the invention provides a method for identifying compositions that modulate the activity or expression of a polynucleotide or polypeptide of the invention. For example, a test compound, whether a small or large molecule, is placed in contact with a cell,

plant (or plant tissue or explant), or composition comprising the polynucleotide or polypeptide of interest and a resulting effect on the cell, plant, (or tissue or explant) or composition is evaluated by monitoring, either directly or indirectly, one or more of: expression level of the polynucleotide or polypeptide, activity (or modulation of the activity) of the polynucleotide or polypeptide. In  
5 some cases, an alteration in a plant phenotype can be detected following contact of a plant (or plant cell, or tissue or explant) with the putative modulator, e.g., by modulation of expression or activity of a polynucleotide or polypeptide of the invention.

### SUBSEQUENCES

10 Also contemplated are uses of polynucleotides, also referred to herein as oligonucleotides, typically having at least 12 bases, preferably at least 15, more preferably at least 20, 30, or 50 bases, which hybridize under at least highly stringent (or ultra-high stringent or ultra-ultra- high stringent conditions) conditions to a polynucleotide sequence described above. The polynucleotides may be used as probes, primers, sense and antisense agents, and the like,  
15 according to methods as noted *supra*.

Subsequences of the polynucleotides of the invention, including polynucleotide fragments and oligonucleotides are useful as nucleic acid probes and primers. An oligonucleotide suitable for use as a probe or primer is at least about 15 nucleotides in length, more often at least about 18 nucleotides, often at least about 21 nucleotides, frequently at least about 30 nucleotides,  
20 or about 40 nucleotides, or more in length. A nucleic acid probe is useful in hybridization protocols, e.g., to identify additional polypeptide homologues of the invention, including protocols for microarray experiments. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer  
25 pairs can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods. See Sambrook and Ausubel, *supra*.

In addition, the invention includes an isolated or recombinant polypeptide including a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotides of the invention. For example, such polypeptides, or domains or  
30 fragments thereof, can be used as immunogens, e.g., to produce antibodies specific for the polypeptide sequence, or as probes for detecting a sequence of interest. A subsequence can range in size from about 15 amino acids in length up to and including the full length of the polypeptide.

## PRODUCTION OF TRANSGENIC PLANTS

### Modification of Traits

The polynucleotides of the invention are favorably employed to produce transgenic plants with various traits, or characteristics, that have been modified in a desirable manner, e.g., to improve the environmental stress resistance of a plant. For example, alteration of expression levels or patterns (e.g., spatial or temporal expression patterns) of one or more of the transcription factors (or transcription factor homologues) of the invention, as compared with the levels of the same protein found in a wild type plant, can be used to modify a plant's traits. An illustrative example of trait modification, improved environmental stress tolerance, by altering expression levels of a particular transcription factor is described further in the Examples and the Sequence Listing.

### Antisense and Cosuppression Approaches

In addition to expression of the nucleic acids of the invention as gene replacement or plant phenotype modification nucleic acids, the nucleic acids are also useful for sense and anti-sense suppression of expression, e.g., to down-regulate expression of a nucleic acid of the invention, e.g., as a further mechanism for modulating plant phenotype. That is, the nucleic acids of the invention, or subsequences or anti-sense sequences thereof, can be used to block expression of naturally occurring homologous nucleic acids. A variety of sense and anti-sense technologies are known in the art, e.g., as set forth in Lichtenstein and Nellen (1997) Antisense Technology: A Practical Approach IRL Press at Oxford University, Oxford, England. In general, sense or anti-sense sequences are introduced into a cell, where they are optionally amplified, e.g., by transcription. Such sequences include both simple oligonucleotide sequences and catalytic sequences such as ribozymes.

For example, a reduction or elimination of expression (i.e., a "knock-out") of a transcription factor or transcription factor homologue polypeptide in a transgenic plant, e.g., to modify a plant trait, can be obtained by introducing an antisense construct corresponding to the polypeptide of interest as a cDNA. For antisense suppression, the transcription factor or homologue cDNA is arranged in reverse orientation (with respect to the coding sequence) relative to the promoter sequence in the expression vector. The introduced sequence need not be the full length cDNA or gene, and need not be identical to the cDNA or gene found in the plant type to be transformed. Typically, the antisense sequence need only be capable of hybridizing to the target gene or RNA of interest. Thus, where the introduced sequence is of shorter length, a higher degree of homology to the endogenous transcription factor sequence will be needed for effective antisense suppression. While antisense sequences of various lengths can be utilized, preferably,



the introduced antisense sequence in the vector will be at least 30 nucleotides in length, and improved antisense suppression will typically be observed as the length of the antisense sequence increases. Preferably, the length of the antisense sequence in the vector will be greater than 100 nucleotides. Transcription of an antisense construct as described results in the production of  
5 RNA molecules that are the reverse complement of mRNA molecules transcribed from the endogenous transcription factor gene in the plant cell.

Suppression of endogenous transcription factor gene expression can also be achieved using a ribozyme. Ribozymes are RNA molecules that possess highly specific endoribonuclease activity. The production and use of ribozymes are disclosed in U.S. Patent No.  
10 4,987,071 and U.S. Patent No. 5,543,508. Synthetic ribozyme sequences including antisense RNAs can be used to confer RNA cleaving activity on the antisense RNA, such that endogenous mRNA molecules that hybridize to the antisense RNA are cleaved, which in turn leads to an enhanced antisense inhibition of endogenous gene expression.

Vectors in which RNA encoded by a transcription factor or transcription factor  
15 homologue cDNA is over-expressed can also be used to obtain co-suppression of a corresponding endogenous gene, e.g., in the manner described in U.S. Patent No. 5,231,020 to Jorgensen. Such co-suppression (also termed sense suppression) does not require that the entire transcription factor cDNA be introduced into the plant cells, nor does it require that the introduced sequence be exactly identical to the endogenous transcription factor gene of interest. However, as with  
20 antisense suppression, the suppressive efficiency will be enhanced as specificity of hybridization is increased, e.g., as the introduced sequence is lengthened, and/or as the sequence similarity between the introduced sequence and the endogenous transcription factor gene is increased.

Vectors expressing an untranslatable form of the transcription factor mRNA, e.g., sequences comprising one or more stop codon, or nonsense mutation) can also be used to  
25 suppress expression of an endogenous transcription factor, thereby reducing or eliminating it's activity and modifying one or more traits. Methods for producing such constructs are described in U.S. Patent No. 5,583,021. Preferably, such constructs are made by introducing a premature stop codon into the transcription factor gene. Alternatively, a plant trait can be modified by gene silencing using double-strand RNA (Sharp (1999) Genes and Development 13: 139-141).

30 Another method for abolishing the expression of a gene is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in a transcription factor or transcription factor homologue gene. Plants containing a single transgene insertion

event at the desired gene can be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) Methods in Arabidopsis Research, World Scientific).

Alternatively, a plant phenotype can be altered by eliminating an endogenous gene, such as a transcription factor or transcription factor homologue, e.g., by homologous recombination (Kempin et al. (1997) Nature 389:802).

A plant trait can also be modified by using the cre-lox system (for example, as described in US Pat. No. 5,658,772). A plant genome can be modified to include first and second lox sites that are then contacted with a Cre recombinase. If the lox sites are in the same orientation, the intervening DNA sequence between the two sites is excised. If the lox sites are in the opposite orientation, the intervening sequence is inverted.

The polynucleotides and polypeptides of this invention can also be expressed in a plant in the absence of an expression cassette by manipulating the activity or expression level of the endogenous gene by other means. For example, by ectopically expressing a gene by T-DNA activation tagging (Ichikawa et al. (1997) Nature 390 698-701; Kakimoto et al. (1996) Science 274: 982-985). This method entails transforming a plant with a gene tag containing multiple transcriptional enhancers and once the tag has inserted into the genome, expression of a flanking gene coding sequence becomes deregulated. In another example, the transcriptional machinery in a plant can be modified so as to increase transcription levels of a polynucleotide of the invention (See, e.g., PCT Publications WO 96/06166 and WO 98/53057 which describe the modification of the DNA binding specificity of zinc finger proteins by changing particular amino acids in the DNA binding motif).

The transgenic plant can also include the machinery necessary for expressing or altering the activity of a polypeptide encoded by an endogenous gene, for example by altering the phosphorylation state of the polypeptide to maintain it in an activated state.

Transgenic plants (or plant cells, or plant explants, or plant tissues) incorporating the polynucleotides of the invention and/or expressing the polypeptides of the invention can be produced by a variety of well established techniques as described above. Following construction of a vector, most typically an expression cassette, including a polynucleotide, e.g., encoding a transcription factor or transcription factor homologue, of the invention, standard techniques can be used to introduce the polynucleotide into a plant, a plant cell, a plant explant or a plant tissue of interest. Optionally, the plant cell, explant or tissue can be regenerated to produce a transgenic plant.

The plant can be any higher plant, including gymnosperms, monocotyledonous and dicotyledonous plants. Suitable protocols are available for *Leguminosae* (alfalfa, soybean,

clover, etc.), *Umbelliferae* (carrot, celery, parsnip), *Cruciferae* (cabbage, radish, rapeseed, broccoli, etc.), *Curcubitaceae* (melons and cucumber), *Gramineae* (wheat, corn, rice, barley, millet, etc.), *Solanaceae* (potato, tomato, tobacco, peppers, etc.), and various other crops. See protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture –Crop Species.

- 5 Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

Transformation and regeneration of both monocotyledonous and dicotyledonous plant cells is now routine, and the selection of the most appropriate transformation technique will be determined by the practitioner. The choice of method will vary with the type of plant to be transformed; those skilled in the art will recognize the suitability of particular methods for given plant types. Suitable methods can include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation. Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence.

10  
15

Successful examples of the modification of plant characteristics by transformation with cloned sequences which serve to illustrate the current knowledge in this field of technology, and which are herein incorporated by reference, include: U.S. Patent Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

20

Following transformation, plants are preferably selected using a dominant selectable marker incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic or herbicide.

25

After transformed plants are selected and grown to maturity, those plants showing a modified trait are identified. The modified trait can be any of those traits described above. Additionally, to confirm that the modified trait is due to changes in expression levels or activity of the polypeptide or polynucleotide of the invention can be determined by analyzing mRNA expression using Northern blots, RT-PCR or microarrays, or protein expression using immunoblots or Western blots or gel shift assays.

30

### INTEGRATED SYSTEMS—SEQUENCE IDENTITY

Additionally, the present invention may be an integrated system, computer or computer readable medium that comprises an instruction set for determining the identity of one or more sequences in a database. In addition, the instruction set can be used to generate or identify  
5 sequences that meet any specified criteria. Furthermore, the instruction set may be used to associate or link certain functional benefits, such improved environmental stress tolerance, with one or more identified sequence.

For example, the instruction set can include, e.g., a sequence comparison or other alignment program, e.g., an available program such as, for example, the Wisconsin Package  
10 Version 10.0, such as BLAST, FASTA, PILEUP, FINDPATTERNS or the like (GCG, Madison, WI). Public sequence databases such as GenBank, EMBL, Swiss-Prot and PIR or private sequence databases such as PhytoSeq (Incyte Pharmaceuticals, Palo Alto, CA) can be searched.

Alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment  
15 algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. U.S.A. 85: 2444, by computerized implementations of these algorithms. After alignment, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two sequences over a comparison window to identify and compare local regions of sequence  
20 similarity. The comparison window can be a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 contiguous positions. A description of the method is provided in Ausubel et al., *supra*.

A variety of methods of determining sequence relationships can be used, including manual alignment and computer assisted sequence alignment and analysis. This later  
25 approach is a preferred approach in the present invention, due to the increased throughput afforded by computer assisted methods. As noted above, a variety of computer programs for performing sequence alignment are available, or can be produced by one of skill.

One example algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al. J. Mol. Biol.  
30 215:403-410 (1990). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is

referred to as the neighborhood word score threshold (Altschul et al., *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for

5 nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of

10 one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E)

15 of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see*, e.g., Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided

20 by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence (and, therefore, in this context, homologous) if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, or less than about 0.01, and or

25 even less than about 0.001. An additional example of a useful sequence alignment algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. The program can align, e.g., up to 300 sequences of a maximum length of 5,000 letters.

The integrated system, or computer typically includes a user input interface

30 allowing a user to selectively view one or more sequence records corresponding to the one or more character strings, as well as an instruction set which aligns the one or more character strings with each other or with an additional character string to identify one or more region of sequence similarity. The system may include a link of one or more character strings with a particular

phenotype or gene function. Typically, the system includes a user readable output element which displays an alignment produced by the alignment instruction set.

The methods of this invention can be implemented in a localized or distributed computing environment. In a distributed environment, the methods may implemented on a single  
5 computer comprising multiple processors or on a multiplicity of computers. The computers can be linked, e.g. through a common bus, but more preferably the computer(s) are nodes on a network. The network can be a generalized or a dedicated local or wide-area network and, in certain preferred embodiments, the computers may be components of an intra-net or an internet.

Thus, the invention provides methods for identifying a sequence similar or  
10 homologous to one or more polynucleotides as noted herein, or one or more target polypeptides encoded by the polynucleotides, or otherwise noted herein and may include linking or associating a given plant phenotype or gene function with a sequence. In the methods, a sequence database is provided (locally or across an inter or intra net) and a query is made against the sequence database using the relevant sequences herein and associated plant phenotypes or gene functions.

Any sequence herein can be entered into the database, before or after querying  
15 the database. This provides for both expansion of the database and, if done before the querying step, for insertion of control sequences into the database. The control sequences can be detected by the query to ensure the general integrity of both the database and the query. As noted, the query can be performed using a web browser based interface. For example, the database can be a  
20 centralized public database such as those noted herein, and the querying can be done from a remote terminal or computer across an internet or intranet.

### EXAMPLES

The following examples are intended to illustrate but not limit the present invention.

#### 25 EXAMPLE I. FULL LENGTH GENE IDENTIFICATION AND CLONING

Putative transcription factor sequences (genomic or ESTs) related to known transcription factors were identified in the *Arabidopsis thaliana* GenBank database using the tblastn sequence analysis program using default parameters and a P-value cutoff threshold of -4  
30 or -5 or lower, depending on the length of the query sequence. Putative transcription factor sequence hits were then screened to identify those containing particular sequence strings. If the sequence hits contained such sequence strings, the sequences were confirmed as transcription factors.

Alternatively, *Arabidopsis thaliana* cDNA libraries derived from different tissues or treatments, or genomic libraries were screened to identify novel members of a transcription family using a low stringency hybridization approach. Probes were synthesized using gene specific primers in a standard PCR reaction (annealing temperature 60°C) and labeled with <sup>32</sup>P dCTP using the High Prime DNA Labeling Kit (Boehringer Mannheim). Purified radiolabelled probes were added to filters immersed in Church hybridization medium (0.5 M NaPO<sub>4</sub> pH 7.0, 7% SDS, 1 % w/v bovine serum albumin) and hybridized overnight at 60 °C with shaking. Filters were washed two times for 45 to 60 minutes with 1xSCC, 1% SDS at 60°C.

To identify additional sequence 5' or 3' of a partial cDNA sequence in a cDNA library, 5' and 3' rapid amplification of cDNA ends (RACE) was performed using the Marathon™ cDNA amplification kit (Clontech, Palo Alto, CA). Generally, the method entailed first isolating poly(A) mRNA, performing first and second strand cDNA synthesis to generate double stranded cDNA, blunting cDNA ends, followed by ligation of the Marathon™ Adaptor to the cDNA to form a library of adaptor-ligated ds cDNA.

Gene-specific primers were designed to be used along with adaptor specific primers for both 5' and 3' RACE reactions. Nested primers, rather than single primers, were used to increase PCR specificity. Using 5' and 3' RACE reactions, 5' and 3' RACE fragments were obtained, sequenced and cloned. The process can be repeated until 5' and 3' ends of the full-length gene were identified. Then the full-length cDNA was generated by PCR using primers specific to 5' and 3' ends of the gene by end-to-end PCR.

#### EXAMPLE II. CONSTRUCTION OF EXPRESSION VECTORS

The sequence was amplified from a genomic or cDNA library using primers specific to sequences upstream and downstream of the coding region. The expression vector was pMEN20 or pMEN65, which are both derived from pMON316 (Sanders et al, (1987) Nucleic Acids Research 15:1543-58) and contain the CaMV 35S promoter to express transgenes. To clone the sequence into the vector, both pMEN20 and the amplified DNA fragment were digested separately with SalI and NotI restriction enzymes at 37° C for 2 hours. The digestion products were subject to electrophoresis in a 0.8% agarose gel and visualized by ethidium bromide staining. The DNA fragments containing the sequence and the linearized plasmid were excised and purified by using a Qiaquick gel extraction kit (Qiagen, CA). The fragments of interest were ligated at a ratio of 3:1 (vector to insert). Ligation reactions using T4 DNA ligase (New England Biolabs, MA) were carried out at 16° C for 16 hours. The ligated DNAs were transformed into

competent cells of the *E. coli* strain DH5alpha by using the heat shock method. The transformations were plated on LB plates containing 50 mg/l kanamycin (Sigma).

Individual colonies were grown overnight in five milliliters of LB broth containing 50 mg/l kanamycin at 37° C. Plasmid DNA was purified by using Qiaquick Mini  
5 Prep kits (Qiagen, CA).

### EXAMPLE III. TRANSFORMATION OF *AGROBACTERIUM* WITH THE EXPRESSION VECTOR

After the plasmid vector containing the gene was constructed, the vector was used to transform *Agrobacterium tumefaciens* cells expressing the gene products. The stock of  
10 *Agrobacterium tumefaciens* cells for transformation were made as described by Nagel et al. (1990) FEMS Microbiol Letts. 67: 325-328. *Agrobacterium* strain ABI was grown in 250 ml LB medium (Sigma) overnight at 28°C with shaking until an absorbance ( $A_{600}$ ) of 0.5 – 1.0 was reached. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4° C. Cells were then resuspended in 250 µl chilled buffer (1 mM HEPES, pH adjusted to 7.0 with KOH). Cells were  
15 centrifuged again as described above and resuspended in 125 µl chilled buffer. Cells were then centrifuged and resuspended two more times in the same HEPES buffer as described above at a volume of 100 µl and 750 µl, respectively. Resuspended cells were then distributed into 40 µl aliquots, quickly frozen in liquid nitrogen, and stored at -80° C.

*Agrobacterium* cells were transformed with plasmids prepared as described  
20 above following the protocol described by Nagel et al. For each DNA construct to be transformed, 50 – 100 ng DNA (generally resuspended in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was mixed with 40 µl of *Agrobacterium* cells. The DNA/cell mixture was then transferred to a chilled cuvette with a 2mm electrode gap and subject to a 2.5 kV charge dissipated at 25 µF and 200 µF using a Gene Pulser II apparatus (Bio-Rad). After electroporation, cells were  
25 immediately resuspended in 1.0 ml LB and allowed to recover without antibiotic selection for 2 – 4 hours at 28° C in a shaking incubator. After recovery, cells were plated onto selective medium of LB broth containing 100 µg/ml spectinomycin (Sigma) and incubated for 24-48 hours at 28° C. Single colonies were then picked and inoculated in fresh medium. The presence of the plasmid construct was verified by PCR amplification and sequence analysis.

### 30 EXAMPLE IV. TRANSFORMATION OF *ARABIDOPSIS* PLANTS WITH *AGROBACTERIUM TUMEFACIENS* WITH EXPRESSION VECTOR

After transformation of *Agrobacterium tumefaciens* with plasmid vectors containing the gene, single *Agrobacterium* colonies were identified, propagated, and used to



transform *Arabidopsis* plants. Briefly, 500 ml cultures of LB medium containing 50 mg/l kanamycin were inoculated with the colonies and grown at 28° C with shaking for 2 days until an absorbance ( $A_{600}$ ) of > 2.0 is reached. Cells were then harvested by centrifugation at 4,000 x g for 10 min, and resuspended in infiltration medium (1/2 X Murashige and Skoog salts (Sigma), 1 X Gamborg's B-5 vitamins (Sigma), 5.0% (w/v) sucrose (Sigma), 0.044  $\mu$ M benzylamino purine (Sigma), 200  $\mu$ L/L Silwet L-77 (Lehle Seeds) until an absorbance ( $A_{600}$ ) of 0.8 was reached.

Prior to transformation, *Arabidopsis thaliana* seeds (ecotype Columbia) were sown at a density of ~10 plants per 4" pot onto Pro-Mix BX potting medium (Hummert International) covered with fiberglass mesh (18 mm X 16 mm). Plants were grown under continuous illumination (50-75  $\mu$ E/m<sup>2</sup>/sec) at 22-23° C with 65-70% relative humidity. After about 4 weeks, primary inflorescence stems (bolts) are cut off to encourage growth of multiple secondary bolts. After flowering of the mature secondary bolts, plants were prepared for transformation by removal of all siliques and opened flowers.

The pots were then immersed upside down in the mixture of *Agrobacterium* infiltration medium as described above for 30 sec, and placed on their sides to allow draining into a 1' x 2' flat surface covered with plastic wrap. After 24 h, the plastic wrap was removed and pots are turned upright. The immersion procedure was repeated one week later, for a total of two immersions per pot. Seeds were then collected from each transformation pot and analyzed following the protocol described below.

#### 20 EXAMPLE V. IDENTIFICATION OF ARABIDOPSIS PRIMARY TRANSFORMANTS

Seeds collected from the transformation pots were sterilized essentially as follows. Seeds were dispersed into in a solution containing 0.1% (v/v) Triton X-100 (Sigma) and sterile H<sub>2</sub>O and washed by shaking the suspension for 20 min. The wash solution was then drained and replaced with fresh wash solution to wash the seeds for 20 min with shaking. After removal of the second wash solution, a solution containing 0.1% (v/v) Triton X-100 and 70% ethanol (Equistar) was added to the seeds and the suspension was shaken for 5 min. After removal of the ethanol/detergent solution, a solution containing 0.1% (v/v) Triton X-100 and 30% (v/v) bleach (Clorox) was added to the seeds, and the suspension was shaken for 10 min. After removal of the bleach/detergent solution, seeds were then washed five times in sterile distilled H<sub>2</sub>O. The seeds were stored in the last wash water at 4° C for 2 days in the dark before being plated onto antibiotic selection medium (1 X Murashige and Skoog salts (pH adjusted to 5.7 with 1M KOH), 1 X Gamborg's B-5 vitamins, 0.9% phytagar (Life Technologies), and 50 mg/l kanamycin). Seeds were germinated under continuous illumination (50-75  $\mu$ E/m<sup>2</sup>/sec) at 22-23°

C. After 7-10 days of growth under these conditions, kanamycin resistant primary transformants (T<sub>1</sub> generation) were visible and obtained. These seedlings were transferred first to fresh selection plates where the seedlings continued to grow for 3-5 more days, and then to soil (Pro-Mix BX potting medium).

- 5                    Primary transformants were crossed and progeny seeds (T<sub>2</sub>) collected; kanamycin resistant seedlings were selected and analyzed. The expression levels of the recombinant polynucleotides in the transformants varies from about a 5% expression level increase to a least a 100% expression level increase. Similar observations are made with respect to polypeptide level expression.

10

#### EXAMPLE VI. IDENTIFICATION OF ARABIDOPSIS PLANTS WITH TRANSCRIPTION FACTOR GENE KNOCKOUTS

- 15                    The screening of insertion mutagenized *Arabidopsis* collections for null mutants in a known target gene was essentially as described in Krysan et al (1999) Plant Cell 11:2283-2290. Briefly, gene-specific primers, nested by 5-250 base pairs to each other, were designed from the 5' and 3' regions of a known target gene. Similarly, nested sets of primers were also created specific to each of the T-DNA or transposon ends (the "right" and "left" borders). All possible combinations of gene specific and T-DNA/transposon primers were used to detect by
- 20                    PCR an insertion event within or close to the target gene. The amplified DNA fragments were then sequenced which allows the precise determination of the T-DNA/transposon insertion point relative to the target gene. Insertion events within the coding or intervening sequence of the genes were deconvoluted from a pool comprising a plurality of insertion events to a single unique mutant plant for functional characterization. The method is described in more detail in Yu and
- 25                    Adam, US Application Serial No. 09/177,733 filed October 23, 1998.

#### EXAMPLE VII. IDENTIFICATION OF ENVIRONMENTAL STRESS TOLERANCE PHENOTYPE IN OVEREXPRESSOR OR GENE KNOCKOUT PLANTS

- 30                    Experiments were performed to identify those transformants or knockouts that exhibited an improved environmental stress tolerance. For such studies, the transformants were exposed to a variety of environmental stresses. Plants were exposed to chilling stress (6 hour exposure to 4-8° C ), heat stress (6 hour exposure to 32-37° C), high salt stress (6 hour exposure to 200 mM NaCl), drought stress (168 hours after removing water from trays), osmotic stress (6 hour exposure to 3 M mannitol), or nutrient limitation (nitrogen, phosphate, and potassium) (Nitrogen: all components of MS medium remained constant except N was reduced to 20mg/L of

NH<sub>4</sub>NO<sub>3</sub>, or Phosphate: All components of MS medium except KH<sub>2</sub>PO<sub>4</sub>, which was replaced by K<sub>2</sub>SO<sub>4</sub>, Potassium: All components of MS medium except removal of KNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>, which were replaced by NaH<sub>4</sub>PO<sub>4</sub>).

- 5 Table 3 shows the phenotypes observed for particular overexpressor or knockout plants and provides the SEQ ID No., the internal reference code (GID), whether a knockout or overexpressor plant was analyzed and the observed phenotype.

**Table 3**

SEQ ID No.	GID	Knockout (KO) or overexpressor (OX)	Phenotype observed
1	G22	OE	Increased tolerance to high salt
3	G188	KO	Better germination under osmotic stress
5	G225	OE	Increased tolerance to nitrogen-limited medium
7	G226	OE	Increased tolerance to nitrogen-limited medium
9	G256	OE	Better germination and growth in cold
11	G419	OE	Increased tolerance to potassium-free medium
13	G464	OE	Better germination and growth in heat
15	G482	OE	Increased tolerance to high salt
17	G502	KO	Increased sensitivity to osmotic stress
19	G526	OE	Increased sensitivity to osmotic stress
21	G545	OE	Susceptible to high salt
23	G561	OE	Increased tolerance to potassium-free medium
25	G664	OE	Better germination and growth in cold
27	G682	OE	Better germination and growth in heat
29	G911	OE	Increased growth on potassium-free medium
31	G964	OE	Better germination and growth in heat
33	G394	OE	More sensitive to chilling
35	G489	OE	Increased tolerance to osmotic stress

- 10 For a particular overexpressor that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with a decreased expression of the particular transcription factor. For a particular knockout that shows a decreased tolerance to an environmental stress, it may be more useful to select a plant with an increased expression of the particular transcription factor.

#### EXAMPLE VIII. IDENTIFICATION OF HOMOLOGOUS SEQUENCES

- 15 Homologous sequences from *Arabidopsis* and plant species other than *Arabidopsis* were identified using database sequence search tools, such as the Basic Local Alignment Search Tool (BLAST) (Altschul et al. (1990) *J. Mol. Biol.* 215:403-410; and Altschul et al. (1997) *Nucl. Acid Res.* 25: 3389-3402). The tblastx sequence analysis programs were employed using the

BLOSUM-62 scoring matrix (Henikoff, S. and Henikoff, J. G. (1992) Proc. Natl. Acad. Sci. USA 89: 10915-10919).

Identified *Arabidopsis* homologous sequences are provided in Figure 2 and included in the Sequence Listing. The percent sequence identity among these sequences is as low as 47% sequence identity. Additionally, the entire NCBI GenBank database was filtered for sequences from all plants except *Arabidopsis thaliana* by selecting all entries in the NCBI GenBank database associated with NCBI taxonomic ID 33090 (Viridiplantae; all plants) and excluding entries associated with taxonomic ID 3701 (*Arabidopsis thaliana*). These sequences were compared to sequences representing genes of SEQ IDs Nos. 1-54 on 9/26/2000 using the Washington University TBLASTX algorithm (version 2.0a19MP). For each gene of SEQ IDs Nos. 1-54, individual comparisons were ordered by probability score (P-value), where the score reflects the probability that a particular alignment occurred by chance. For example, a score of  $3.6 \times 10^{-40}$  is  $3.6 \times 10^{-40}$ . For up to ten species, the gene with the lowest P-value (and therefore the most likely homolog) is listed in Figure 3.

In addition to P-values, comparisons were also scored by percentage identity. Percentage identity reflects the degree to which two segments of DNA or protein are identical over a particular length. The ranges of percent identity between the non-*Arabidopsis* genes shown in Figure 3 and the *Arabidopsis* genes in the sequence listing are: SEQ ID No. 1: 53%-67%; SEQ ID No. 3: 38%-76%; SEQ ID No. 5: 34%-67%; SEQ ID No. 7: 50%-69%; SEQ ID No. 9: 32%-91%; SEQ ID No. 11: 48%-66%; SEQ ID No. 13: 34%-60%; SEQ ID No. 15: 58%-81%; SEQ ID No. 17: 65%-94%; SEQ ID No. 19: 72%-83%; SEQ ID No. 21: 52%-64%; SEQ ID No. 23: 40%-89%; SEQ ID No. 25: 86%-97%; SEQ ID No. 27: 41%-75%; SEQ ID No. 29: 29%-72%; SEQ ID No. 31: 49%-70%; SEQ ID No. 33: 56%-86%; SEQ ID No. 35: 61%-84%; SEQ ID No. 37: 40%-58%; SEQ ID No. 39: 63%-87%; SEQ ID No. 41: 51%-88%; SEQ ID No. 43: 80%-90%; SEQ ID No. 45: 79%-90%; SEQ ID No. 47: 30%-58%; SEQ ID No. 49: 52%-62%; SEQ ID No. 51: 55%-73% and SEQ ID No. 53: 44%-80%.

The polynucleotides and polypeptides in the Sequence Listing and the identified homologous sequences may be stored in a computer system and have associated or linked with the sequences a function, such as that the polynucleotides and polypeptides are useful for modifying the environmental stress tolerance of a plant.

All references, publications, patents and other documents herein are incorporated by reference in their entirety for all purposes. Although the invention has been described with

reference to the embodiments and examples above, it should be understood that various modifications can be made without departing from the spirit of the invention.

What is claimed is:

1. A transgenic plant with modified environmental stress tolerance, which plant comprises a recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:
  - 5 (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;
  - (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
  - (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-10 1, where N=1-27, or a complementary nucleotide sequence thereof;
  - (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
  - (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
  - (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of 15 any of (a)-(e);
  - (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
  - (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence 20 of any of (a)-(g);
  - (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide sequence of any of (a)-(g);
  - (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
  - 25 (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and
  - (l) a nucleotide sequence which encodes a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.
- 30 2. The transgenic plant of claim 1, further comprising a constitutive, inducible, or tissue-active promoter operably linked to said nucleotide sequence.
3. The transgenic plant of claim 1, wherein the plant is selected from the group consisting of: soybean, wheat, corn, potato, cotton, rice, oilseed rape, sunflower, alfalfa, sugarcane, turf,

banana, blackberry, blueberry, strawberry, raspberry, cantaloupe, carrot, cauliflower, coffee, cucumber, eggplant, grapes, honeydew, lettuce, mango, melon, onion, papaya, peas, peppers, pineapple, spinach, squash, sweet corn, tobacco, tomato, watermelon, rosaceous fruits, and vegetable brassicas.

5

4. An isolated or recombinant polynucleotide comprising a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide comprising a sequence selected from SEQ ID Nos. 2N, where N=1-27, or a complementary nucleotide sequence thereof;
- 10 (b) a nucleotide sequence encoding a polypeptide comprising a conservatively substituted variant of a polypeptide of (a);
- (c) a nucleotide sequence comprising a sequence selected from those of SEQ ID Nos. 2N-1, where N=1-27, or a complementary nucleotide sequence thereof;
- (d) a nucleotide sequence comprising silent substitutions in a nucleotide sequence of (c);
- 15 (e) a nucleotide sequence which hybridizes under stringent conditions to a nucleotide sequence of one or more of: (a), (b), (c), or (d);
- (f) a nucleotide sequence comprising at least 15 consecutive nucleotides of a sequence of any of (a)-(e);
- (g) a nucleotide sequence comprising a subsequence or fragment of any of (a)-(f), which
- 20 subsequence or fragment encodes a polypeptide that modifies a plant's environmental stress tolerance;
- (h) a nucleotide sequence having at least 30% sequence identity to a nucleotide sequence of any of (a)-(g);
- (i) a nucleotide sequence having at least 60% identity sequence identity to a nucleotide
- 25 sequence of any of (a)-(g);
- (j) a nucleotide sequence which encodes a polypeptide having at least 30% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27;
- (k) a nucleotide sequence which encodes a polypeptide having at least 60% identity sequence identity to a polypeptide of SEQ ID Nos. 2N, where N=1-27; and
- 30 (l) a nucleotide sequence which encodes a conserved domain of a polypeptide having at least 65% sequence identity to a conserved domain of a polypeptide of SEQ ID Nos. 2N, where N=1-27.

5. The isolated or recombinant polynucleotide of claim 4, further comprising a constitutive, inducible, or tissue-active promoter operably linked to the nucleotide sequence.
6. A cloning or expression vector comprising the isolated or recombinant polynucleotide of claim 4.
7. A cell comprising the cloning or expression vector of claim 6.
8. A transgenic plant comprising the isolated or recombinant polynucleotide of claim 4.
9. A composition produced by one or more of:  
(a) incubating one or more polynucleotide of claim 4 with a nuclease;  
(b) incubating one or more polynucleotide of claim 4 with a restriction enzyme;  
(c) incubating one or more polynucleotide of claim 4 with a polymerase;  
(d) incubating one or more polynucleotide of claim 4 with a polymerase and a primer;  
(e) incubating one or more polynucleotide of claim 4 with a cloning vector, or  
(f) incubating one or more polynucleotide of claim 4 with a cell.
10. A composition comprising two or more different polynucleotides of claim 4.
11. An isolated or recombinant polypeptide comprising a subsequence of at least about 15 contiguous amino acids encoded by the recombinant or isolated polynucleotide of claim 4.
12. A plant ectopically expressing an isolated polypeptide of claim 11.
13. A method for producing a plant having a modified environmental stress tolerance, the method comprising altering the expression of the isolated or recombinant polynucleotide of claim 4 or the expression levels or activity of a polypeptide of claim 11 in a plant, thereby producing a modified plant, and selecting the modified plant for improved environmental stress tolerance thereby providing the modified plant with a modified environmental stress tolerance.
14. The method of claim 13, wherein the polynucleotide is a polynucleotide of claim 4.



15. A method of identifying a factor that is modulated by or interacts with a polypeptide encoded by a polynucleotide of claim 4, the method comprising:

- (a) expressing a polypeptide encoded by the polynucleotide in a plant; and
- (b) identifying at least one factor that is modulated by or interacts with the polypeptide.

5

16. The method of claim 15, wherein the identifying is performed by detecting binding by the polypeptide to a promoter sequence, or detecting interactions between an additional protein and the polypeptide in a yeast two hybrid system.

10 17. The method of claim 15, wherein the identifying is performed by detecting expression of a factor by hybridization to a microarray, subtractive hybridization or differential display.

18. A method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest, the method comprising:

- 15 (a) placing the molecule in contact with a plant comprising the polynucleotide or polypeptide encoded by the polynucleotide of claim 4; and,
- (b) monitoring one or more of:
  - (i) expression level of the polynucleotide in the plant;
  - (ii) expression level of the polypeptide in the plant;
  - 20 (iii) modulation of an activity of the polypeptide in the plant; or
  - (iv) modulation of an activity of the polynucleotide in the plant.

19. An integrated system, computer or computer readable medium comprising one or more character strings corresponding to a polynucleotide of claim 4, or to a polypeptide encoded by the polynucleotide.

25

20. The integrated system, computer or computer readable medium of claim 19, further comprising a link between said one or more sequence strings to a modified plant environmental stress tolerance phenotype.

30

21. A method of identifying a sequence similar or homologous to one or more polynucleotides of claim 4, or one or more polypeptides encoded by the polynucleotides, the method comprising:

- (a) providing a sequence database; and,

(b) querying the sequence database with one or more target sequences corresponding to the one or more polynucleotides or to the one or more polypeptides to identify one or more sequence members of the database that display sequence similarity or homology to one or more of the one or more target sequences.

5

22. The method of claim 21, wherein the querying comprises aligning one or more of the target sequences with one or more of the one or more sequence members in the sequence database.

10 23. The method of claim 21, wherein the querying comprises identifying one or more of the one or more sequence members of the database that meet a user-selected identity criteria with one or more of the target sequences.

15 24. The method of claim 21, further comprising linking the one or more of the polynucleotides of claim 4, or encoded polypeptides, to a modified plant environmental stress tolerance phenotype.

25. A plant comprising altered expression levels of an isolated or recombinant polynucleotide of claim 4.

20

26. A plant comprising altered expression levels or the activity of an isolated or recombinant polypeptide of claim 11.

27. A plant lacking a nucleotide sequence encoding a polypeptide of claim 11.

25

Figure 1

SEQ ID No.	GID	cDNA or protein	conserved domain
1	G22	cDNA	
2	G22	protein	89-157
3	G188	cDNA	
4	G188	protein	175-222
5	G225	cDNA	
6	G225	protein	39-76
7	G226	cDNA	
8	G226	protein	28-78
9	G256	cDNA	
10	G256	protein	13-115
11	G419	cDNA	
12	G419	protein	392-452
13	G464	cDNA	
14	G464	protein	7-15,70-80,125-158,183-219
15	G482	cDNA	
16	G482	protein	25-116
17	G502	cDNA	
18	G502	protein	10-155
19	G526	cDNA	
20	G526	protein	21-149
21	G545	cDNA	
22	G545	protein	82-102, 136-154
23	G561	cDNA	
24	G561	protein	248-308
25	G664	cDNA	
26	G664	protein	13-116
27	G682	cDNA	
28	G682	protein	22-53
29	G911	cDNA	
30	G911	protein	86-129
31	G964	cDNA	
32	G964	protein	126-186
33	G394	cDNA	
34	G394	protein	121-182
35	G489	cDNA	
36	G489	protein	57-156

Figure 2

SEQ ID No.	GID	homolog	cDNA or protein	conserved domain
37	G463	homolog of G464	cDNA	
38	G463	homolog of G464	protein	14-23, 77-88, 130-146, 194-227
39	G767	homolog of G502	cDNA	
40	G767	homolog of G502	protein	8-158
41	G765	homolog of G526	cDNA	
42	G765	homolog of G526	protein	23-167
43	G197	homolog of G664	cDNA	
44	G197	homolog of G664	protein	14-119
45	G255	homolog of G664	cDNA	
46	G255	homolog of G664	protein	14-115
47	G1113	homolog of G911	cDNA	
48	G1113	homolog of G911	protein	85-128
49	G398	homolog of G964	cDNA	
50	G398	homolog of G964	protein	128-191
51	G395	homolog of G394	cDNA	
52	G395	homolog of G394	protein	72-135
53	G393	homolog of G394	cDNA	
54	G393	homolog of G394	protein	106-169

Figure 3A

SEQ ID No.	GID	Genbank NID	P-value	Species
1	G22	790359	1.00E-45	Nicotiana tabacum
1	G22	3342210	6.60E-45	Lycopersicon esculentum
1	G22	6654776	1.60E-44	Medicago truncatula
1	G22	8809570	5.80E-44	Nicotiana glauca
1	G22	7627061	2.40E-39	Gossypium arboreum
1	G22	7324479	9.50E-36	Lycopersicon pennellii
1	G22	8980312	4.30E-31	Catharanthus roseus
1	G22	7528275	1.20E-30	Mesembryanthemum crystallinum
1	G22	6478844	4.60E-28	Matricaria chamomilla
1	G22	6847348	5.90E-26	Glycine max
3	G188	7779802	5.20E-36	Lotus japonicus
3	G188	7284340	2.10E-34	Glycine max
3	G188	9361307	1.20E-27	Triticum aestivum
3	G188	7340336	1.10E-22	Oryza sativa
3	G188	6529152	3.60E-22	Lycopersicon esculentum
3	G188	8748477	7.70E-21	Medicago truncatula
3	G188	5456433	7.10E-14	Zea mays
3	G188	9302479	1.60E-12	Sorghum bicolor
3	G188	6696287	4.10E-12	Pinus taeda
3	G188	562242	9.00E-12	Brassica rapa
5	G225	4396287	4.40E-16	Glycine max
5	G225	309571	0.00029	Zea mays
5	G225	3857004	0.001	Populus tremula x Populus tremuloides
5	G225	9410205	0.019	Triticum aestivum
5	G225	9426190	0.025	Triticum turgidum subsp. durum
5	G225	8382118	0.046	Gossypium arboreum
5	G225	6782756	0.27	Oryza sativa
5	G225	7721017	0.4	Lotus japonicus
5	G225	6020136	0.47	Pinus taeda
5	G225	2921331	0.48	Gossypium hirsutum
7	G226	4396287	5.10E-15	Glycine max
7	G226	9410205	1.50E-05	Triticum aestivum
7	G226	3857004	0.11	Populus tremula x Populus tremuloides
7	G226	2428139	0.35	Oryza sativa
9	G256	1430847	1.30E-72	Lycopersicon esculentum
9	G256	9252441	1.20E-65	Solanum tuberosum
9	G256	8380712	2.20E-58	Gossypium arboreum
9	G256	8172976	1.60E-54	Medicago truncatula
9	G256	9205295	1.30E-44	Glycine max
9	G256	20562	6.40E-40	Petunia x hybrida
9	G256	4886263	4.40E-37	Antirrhinum majus
9	G256	6552360	5.00E-36	Nicotiana tabacum
9	G256	2312003	1.20E-35	Oryza sativa
9	G256	5268628	5.20E-35	Zea mays
11	G419	7239156	2.60E-59	Malus x domestica
11	G419	5278451	9.00E-58	Lycopersicon esculentum
11	G419	9205496	1.30E-55	Glycine max
11	G419	7628137	9.30E-51	Gossypium arboreum
11	G419	6069643	9.50E-51	Oryza sativa
11	G419	7562931	9.80E-45	Medicago truncatula
11	G419	7322293	2.30E-37	Lycopersicon hirsutum
11	G419	8404716	1.10E-29	Hordeum vulgare
11	G419	7217755	1.40E-29	Sorghum bicolor

Figure 3B

SEQ ID No.	GID	Genbank NID	P-value	Species
11	G419	9428023	4.60E-28	Triticum aestivum
13	G464	6527230	3.60E-31	Lycopersicon esculentum
13	G464	9305572	1.10E-22	Sorghum bicolor
13	G464	6604917	6.70E-22	Medicago truncatula
13	G464	5058123	2.30E-21	Glycine max
13	G464	3760881	1.20E-19	Oryza sativa
13	G464	5044476	1.20E-17	Gossypium hirsutum
13	G464	9412603	6.40E-15	Triticum aestivum
13	G464	7777277	3.20E-13	Lotus japonicus
13	G464	9410371	1.70E-11	Hordeum vulgare
13	G464	7624108	2.10E-10	Gossypium arboreum
15	G482	7691987	5.50E-50	Glycine max
15	G482	7781090	1.30E-48	Lotus japonicus
15	G482	7409616	1.10E-47	Lycopersicon esculentum
15	G482	9416562	4.40E-46	Triticum aestivum
15	G482	22379	2.30E-44	Zea mays
15	G482	7501372	7.70E-44	Gossypium arboreum
15	G482	7765436	8.40E-42	Medicago truncatula
15	G482	5044464	1.20E-40	Gossypium hirsutum
15	G482	9441376	9.20E-40	Chlamydomonas reinhardtii
15	G482	8071558	3.50E-39	Solanum tuberosum
17	G502	6730941	1.60E-91	Oryza sativa
17	G502	7765679	1.60E-82	Medicago truncatula
17	G502	7502501	7.30E-80	Gossypium arboreum
17	G502	5510359	8.30E-77	Glycine max
17	G502	5601137	8.70E-76	Lycopersicon esculentum
17	G502	9302206	1.40E-73	Sorghum bicolor
17	G502	4089948	3.40E-50	Brassica napus
17	G502	8329134	7.90E-49	Mesembryanthemum crystallinum
17	G502	7723564	8.60E-49	Lotus japonicus
17	G502	4218534	1.80E-48	Triticum sp.
19	G526	5049217	3.40E-61	Gossypium hirsutum
19	G526	6066594	1.50E-55	Petunia x hybrida
19	G526	4384535	1.50E-54	Lycopersicon esculentum
19	G526	6454868	6.60E-54	Glycine max
19	G526	4977542	4.70E-52	Oryza sativa
19	G526	5343151	7.00E-51	Zea mays
19	G526	9361647	5.10E-50	Triticum aestivum
19	G526	6799764	4.30E-48	Medicago truncatula
19	G526	8708684	1.80E-47	Hordeum vulgare
19	G526	4218536	3.60E-47	Triticum sp.
21	G545	4666359	8.30E-55	Datisca glomerata
21	G545	7228328	3.70E-52	Medicago sativa
21	G545	1763062	1.30E-51	Glycine max
21	G545	7206360	3.10E-44	Medicago truncatula
21	G545	7626808	9.60E-40	Gossypium arboreum
21	G545	439492	3.90E-39	Petunia x hybrida
21	G545	4382658	1.70E-38	Lycopersicon esculentum
21	G545	8486215	8.70E-38	Euphorbia esula
21	G545	7322653	6.80E-37	Lycopersicon hirsutum
21	G545	7785845	1.10E-33	Lotus japonicus
23	G561	2995461	5.60E-86	Sinapis alba
23	G561	633153	6.50E-83	Brassica napus

Figure 3C

SEQ ID No.	GID	Genbank NID	P-value	Species
23	G561	1033058	5.90E-65	Raphanus sativus
23	G561	2815304	2.10E-35	Spinacia oleracea
23	G561	1498300	1.60E-34	Petroselinum crispum
23	G561	169958	8.10E-32	Glycine max
23	G561	5381310	2.20E-30	Catharanthus roseus
23	G561	1155053	9.70E-28	Phaseolus vulgaris
23	G561	728627	1.90E-27	Nicotiana tabacum
23	G561	7565950	1.40E-21	Medicago truncatula
25	G664	1167483	4.90E-81	Lycopersicon esculentum
25	G664	7765706	6.30E-69	Medicago truncatula
25	G664	19052	9.30E-68	Hordeum vulgare
25	G664	7626566	4.00E-67	Gossypium arboreum
25	G664	5050757	2.60E-66	Gossypium hirsutum
25	G664	6850206	6.90E-66	Oryza sativa
25	G664	6667606	2.20E-63	Glycine max
25	G664	517492	9.30E-62	Zea mays
25	G664	9302672	1.50E-59	Sorghum bicolor
25	G664	5860031	9.20E-58	Pinus taeda
27	G682	309571	4.40E-08	Zea mays
27	G682	4396287	1.10E-05	Glycine max
27	G682	3857004	0.00051	Populus tremula x Populus tremuloides
27	G682	9410205	0.00085	Triticum aestivum
27	G682	8382118	0.0079	Gossypium arboreum
27	G682	2428139	0.017	Oryza sativa
27	G682	7339148	0.13	Lycopersicon esculentum
27	G682	9302672	0.32	Sorghum bicolor
27	G682	5048991	0.39	Gossypium hirsutum
27	G682	6555777	0.46	Pinus taeda
29	G911	4090113	6.10E-51	Brassica napus
29	G911	5893315	7.70E-25	Lycopersicon esculentum
29	G911	5048452	3.10E-23	Gossypium hirsutum
29	G911	9440241	1.90E-21	Glycine max
29	G911	6917169	1.80E-11	Lycopersicon pennellii
29	G911	9297970	3.20E-11	Sorghum bicolor
29	G911	7137594	4.90E-11	Zea mays
29	G911	9278447	4.60E-10	Lotus japonicus
29	G911	7560271	7.20E-10	Medicago truncatula
29	G911	5043346	4.50E-09	Sorghum halepense
31	G964	7624806	3.30E-72	Gossypium arboreum
31	G964	1234899	9.10E-66	Glycine max
31	G964	1149534	1.50E-61	Pimpinella brachycarpa
31	G964	8919872	3.40E-51	Capsella rubella
31	G964	992597	6.70E-51	Lycopersicon esculentum
31	G964	1235564	1.50E-38	Oryza sativa
31	G964	6605613	3.00E-32	Medicago truncatula
31	G964	1032371	4.50E-28	Helianthus annuus
31	G964	3868846	2.80E-25	Ceratopteris richardii
31	G964	8088109	6.40E-22	Sorghum bicolor
33	G394	8670502	7.90E-59	Glycine max
33	G394	3171738	2.00E-54	Cratostigma plantagineum
33	G394	1032371	1.10E-50	Helianthus annuus
33	G394	7624806	4.30E-47	Gossypium arboreum
33	G394	1160483	2.10E-46	Pimpinella brachycarpa

Figure 3D

SEQ ID No.	GID	Genbank NID	P-value	Species
33	G394	3868846	4.20E-45	Ceratopteris richardii
33	G394	992597	1.10E-44	Lycopersicon esculentum
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35	G489	5257255	8.60E-43	Oryza sativa
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37	G463	9412603	1.70E-17	Triticum aestivum
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37	G463	8547152	3.20E-16	Nicotiana tabacum
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39	G767	4218534	4.30E-51	Triticum sp.
39	G767	6732157	4.30E-51	Triticum monococcum
39	G767	9412602	6.90E-47	Triticum aestivum
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41	G765	6454868	8.50E-56	Glycine max
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41	G765	4977542	2.00E-51	Oryza sativa
41	G765	4218536	2.00E-50	Triticum sp.
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41	G765	9361647	4.50E-49	Triticum aestivum
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43	G197	6850206	1.10E-61	Oryza sativa
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Figure 3E

SEQ ID No.	GID	Genbank NID	P-value	Species
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45	G255	7721017	2.60E-55	Lotus japonicus
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47	G1113	5048452	6.80E-12	Gossypium hirsutum
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49	G398	3171738	1.00E-27	Craterostigma plantagineum
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51	G395	8919872	7.40E-41	Capsella rubella
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51	G395	1032371	3.30E-31	Helianthus annuus
53	G393	8670502	3.60E-55	Glycine max
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53	G393	8919872	2.50E-35	Capsella rubella
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53	G393	3171738	1.50E-31	Craterostigma plantagineum

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Riechmann, Jose Luis  
Heard, Jacqueline  
Ratcliffe, Oliver  
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20 25 30

Ile Glu Trp Glu Phe Ile Ser Met Thr Glu Gln Glu Glu Asp Leu Ile



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## MBI16 Sequence Listing.ST25

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Gln Trp Glu Lys Lys Leu Gln Thr Asp Ile Asn Met Ala Lys Gln Ala			
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Leu Phe Gln Ala Leu Ser Leu Asp Gln Pro Ser Ser Leu Ile Pro Pro			
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Asp Pro Asp Ser Pro Lys Pro His His His Ser Thr Thr Thr Tyr Ala			
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Ser Ser Thr Asp Asn Ile Ser Lys Leu Gln Asn Trp Thr Ser Ser			
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Ser Ser Ser Lys Pro Asn Thr Ser Ser Val Ser Asn Asn Arg Ser Ser			
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agc ccc ggt gaa gga gga ctt ttt gat cat cac tct ttg ttc tca tcg			1070
Ser Pro Gly Glu Gly Gly Leu Phe Asp His His Ser Leu Phe Ser Ser			
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Asn Ser Glu Ser Gly Ser Val Asp Glu Lys Leu Asn Leu Met Ser Glu			
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aca agc atg ttc aaa ggt gag agc aag cca gac ata gac atg gaa gct			1166
Thr Ser Met Phe Lys Gly Glu Ser Lys Pro Asp Ile Asp Met Glu Ala			
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Thr Pro Thr Thr Thr Thr Thr Thr Thr Asp Asp Gln Gly Ser Leu Ser			
290	295	300	
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Leu Ile Glu Lys Trp Leu Phe Asp Asp Gln Gly Leu Val Gln Cys Asp			
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Asp Ser Gln Glu Asp Leu Ile Asp Val Ser Leu Glu Glu Leu Lys			
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Gly Pro Gly Asn Trp Arg Ser Val Pro Ala Asn Thr Gly Leu Leu Arg

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## MBI16 Sequence Listing.ST25

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ctttctattt tgtttattgg ttctttttta ctttttcttg gagatgggtt cttgtagatc 300
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gttttcaatt cttgagagac atg gca aga gat cag ttc tat ggt cac aat aac 413
Met Ala Arg Asp Gln Phe Tyr Gly His Asn Asn
1 5 10

cat cat cat caa gag caa caa cat caa atg att aat cag atc caa ggg 461
His His His Gln Glu Gln Gln His Gln Met Ile Asn Gln Ile Gln Gly
15 20 25

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Phe Asp Glu Thr Asn Gln Asn Pro Thr Asp His His His Tyr Asn His
30 35 40

cag atc ttt ggc tca aac tcc aac atg ggt atg atg ata gac ttc tct 557
Gln Ile Phe Gly Ser Asn Ser Asn Met Gly Met Met Ile Asp Phe Ser
45 50 55

aag caa caa cag att agg atg aca agt ggt tgc gat cat cat cat cat 605
Lys Gln Gln Gln Ile Arg Met Thr Ser Gly Ser Asp His His His His
60 65 70 75

cat cat cag aca agt ggt ggt act gat cag aat cag ctt ctg gaa gat 653
His His Gln Thr Ser Gly Gly Thr Asp Gln Asn Gln Leu Leu Glu Asp
80 85 90

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Ser Ser Ser Ala Met Arg Leu Cys Asn Val Asn Asn Asp Phe Pro Ser
95 100 105

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Glu Val Asn Asp Glu Arg Pro Pro Gln Arg Pro Ser Gln Gly Leu Ser
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Leu Ser Leu Ser Ser Ser Asn Pro Thr Ser Ile Ser Leu Gln Ser Phe
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Thr Gln His Gln Asn Leu Gln His Thr Gln Met Met Met Met Met Met
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Asn Ser His His Gln Asn Asn Asn Asn Asn Asn His Gln His His Asn
175 180 185

cat cat cag ttt cag att ggg agt tcc aag tat ttg agt cca gct caa 989
His His Gln Phe Gln Ile Gly Ser Ser Lys Tyr Leu Ser Pro Ala Gln
190 195 200

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## MBI16 Sequence Listing.ST25

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gaa gag tgg gac aca agt cac cac agc aac aat gat caa cat gac caa Glu Glu Trp Asp Thr Ser His His Ser Asn Asn Asp Gln His Asp Gln 240 245 250	1133
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MBI16 Sequence Listing.ST25

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525	530	535	
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Ser Leu Thr Leu Gly Leu Gln Arg Asn Asp Gly Asn Gly Gly Val Ser			
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Gln Leu Leu His Asp Ile Val			
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Gly Gly Thr Asp Gln Asn Gln Leu Leu Glu Asp Ser Ser Ser Ala Met			
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115	120	125	

## MBI16 Sequence Listing.ST25

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 180 185 190  
 Ile Gly Ser Ser Lys Tyr Leu Ser Pro Ala Gln Glu Leu Leu Ser Glu  
 195 200 205  
 Phe Cys Ser Leu Gly Val Lys Glu Ser Asp Glu Glu Val Met Met Met  
 210 215 220  
 Lys His Lys Lys Lys Gln Lys Gly Lys Gln Gln Glu Glu Trp Asp Thr  
 225 230 235 240  
 Ser His His Ser Asn Asn Asp Gln His Asp Gln Ser Ala Thr Thr Ser  
 245 250 255  
 Ser Lys Lys His Val Pro Pro Leu His Ser Leu Glu Phe Met Glu Leu  
 260 265 270  
 Gln Lys Arg Lys Ala Lys Leu Leu Ser Met Leu Glu Glu Leu Lys Arg  
 275 280 285  
 Arg Tyr Gly His Tyr Arg Glu Gln Met Arg Val Ala Ala Ala Ala Phe  
 290 295 300  
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 Ser Arg Ala Met Ser Arg His Phe Arg Cys Leu Lys Asp Gly Leu Val  
 325 330 335  
 Gly Gln Ile Gln Ala Thr Ser Gln Ala Leu Gly Glu Arg Glu Glu Asp  
 340 345 350  
 Asn Arg Ala Val Ser Ile Ala Ala Arg Gly Glu Thr Pro Arg Leu Arg  
 355 360 365  
 Leu Leu Asp Gln Ala Leu Arg Gln Gln Lys Ser Tyr Arg Gln Met Thr  
 370 375 380  
 Leu Val Asp Ala His Pro Trp Arg Pro Gln Arg Gly Leu Pro Glu Arg  
 385 390 395 400  
 Ala Val Thr Thr Leu Arg Ala Trp Leu Phe Glu His Phe Leu His Pro  
 405 410 415  
 Tyr Pro Ser Asp Val Asp Lys His Ile Leu Ala Arg Gln Thr Gly Leu  
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## MBI16 Sequence Listing.ST25

Ser Arg Ser Gln Val Ser Asn Trp Phe Ile Asn Ala Arg Val Arg Leu  
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Trp Lys Pro Met Ile Glu Glu Met Tyr Cys Glu Glu Thr Arg Ser Glu  
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Gln Met Glu Ile Thr Asn Pro Met Met Ile Asp Thr Lys Pro Asp Pro  
 465 470 475 480

Asp Gln Leu Ile Arg Val Glu Pro Glu Ser Leu Ser Ser Ile Val Thr  
 485 490 495

Asn Pro Thr Ser Lys Ser Gly His Asn Ser Thr His Gly Thr Met Ser  
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Leu Gly Ser Thr Phe Asp Phe Ser Leu Tyr Gly Asn Gln Ala Val Thr  
 515 520 525

Tyr Ala Gly Glu Gly Gly Pro Arg Gly Asp Val Ser Leu Thr Leu Gly  
 530 535 540

Leu Gln Arg Asn Asp Gly Asn Gly Gly Val Ser Leu Ala Leu Ser Pro  
 545 550 555 560

Val Thr Ala Gln Gly Gly Gln Leu Phe Tyr Gly Arg Asp His Ile Glu  
 565 570 575

Glu Gly Pro Val Gln Tyr Ser Ala Ser Met Leu Asp Asp Asp Gln Val  
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 Leu Gly Leu Gly Leu Ser Leu Gly Gly Ala Trp Lys Glu Arg Gly  
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MBI16 Sequence Listing.ST25

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Thr	Cys	Arg	Glu	Thr	Val	Lys	Pro	Leu	Arg	Leu	Leu	Asp	Gly	Ser	Ser		
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		20					25						30		

Trp	Lys	Glu	Arg	Gly	Arg	Ile	Leu	Thr	Ala	Lys	Asp	Phe	Pro	Ser	Val
	35						40					45			

## MBI16 Sequence Listing.ST25

Gly Ser Lys Arg Ser Ala Glu Ser Ser Ser His Gln Gly Ala Ser Pro  
50 55 60

Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Leu His Arg  
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85 90 95

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Val Ser Met Lys Val Asn Pro Lys Val Gln Gly Leu Gly Phe Val Lys  
115 120 125

Val Asn Met Asp Gly Val Gly Ile Gly Arg Lys Val Asp Met Arg Ala  
130 135 140

His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu Glu Met Phe Phe  
145 150 155 160

Gly Met Thr Gly Thr Thr Cys Arg Glu Thr Val Lys Pro Leu Arg Leu  
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aatcattgat ggaaatgatt tgaaaaaaga gtaaagtta tttttttatt ccttgtaatt 180  
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Met Gly Asp Ser Asp Arg Asp Ser Gly Gly Gly Gln Asn Gly 10  
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Asn Asn Gln Asn Gly Gln Ser Ser Leu Ser Pro Arg Glu Gln Asp Arg 15 20 25 30  
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Phe Leu Pro Ile Ala Asn Val Ser Arg Ile Met Lys Lys Ala Leu Pro 35 40 45  
gcc aac gcc aag atc tct aaa gat gcc aaa gag acg atg cag gag tgt 373  
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MBI16 Sequence Listing.ST25

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Gln	Lys	Glu	Lys	Arg	Lys	Thr	Ile	Asn	Gly	Asp	Asp	Leu	Leu	Trp	Ala	
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Met	Thr	Thr	Leu	Gly	Phe	Glu	Asp	Tyr	Val	Glu	Pro	Leu	Lys	Val	Tyr	
	95			100						105				110		
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Leu	Gln	Arg	Phe	Arg	Glu	Ile	Glu	Gly	Glu	Arg	Thr	Gly	Leu	Gly	Arg	
			115					120						125		
cca	cag	act	ggt	ggt	gag	gtc	gga	gag	cat	cag	aga	gat	gct	gtc	gga	613
Pro	Gln	Thr	Gly	Gly	Glu	Val	Gly	Glu	His	Gln	Arg	Asp	Ala	Val	Gly	
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Asp	Gly	Gly	Gly	Phe	Tyr	Gly	Gly	Gly	Gly	Gly	Met	Gln	Tyr	His	Gln	
	145					150					155					
cat	cat	cag	ttt	ctt	cac	cag	cag	aac	cat	atg	tat	gga	gcc	aca	ggt	709
His	His	Gln	Phe	Leu	His	Gln	Gln	Asn	His	Met	Tyr	Gly	Ala	Thr	Gly	
	160					165				170						
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Gly	Gly	Ser	Asp	Ser	Gly	Gly	Gly	Ala	Ala	Ser	Gly	Arg	Thr	Arg	Thr	
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Pro Ile Ala Asn Val Ser Arg Ile Met Lys Lys Ala Leu Pro Ala Asn  
 35 40 45

Ala Lys Ile Ser Lys Asp Ala Lys Glu Thr Met Gln Glu Cys Val Ser  
 50 55 60

Glu Phe Ile Ser Phe Val Thr Gly Glu Ala Ser Asp Lys Cys Gln Lys  
 65 70 75 80

Glu Lys Arg Lys Thr Ile Asn Gly Asp Asp Leu Leu Trp Ala Met Thr  
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## MBI16 Sequence Listing.ST25

Thr Leu Gly Phe Glu Asp Tyr Val Glu Pro Leu Lys Val Tyr Leu Gln  
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Arg Phe Arg Glu Ile Glu Gly Glu Arg Thr Gly Leu Gly Arg Pro Gln  
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Thr Gly Gly Glu Val Gly Glu His Gln Arg Asp Ala Val Gly Asp Gly  
 130 135 140

Gly Gly Phe Tyr Gly Gly Gly Gly Gly Met Gln Tyr His Gln His His  
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 Pro Gly Leu Ala Leu Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro  
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 Ser Gly Tyr Trp Lys Ala Thr Gly Ala Asp Lys Pro Ile Gly Leu Pro  
 85 90 95 100  
 aaa ccg gtc gga att aag aaa gct ctt gtt ttc tac gcc ggc aaa gct 571  
 Lys Pro Val Gly Ile Lys Lys Ala Leu Val Phe Tyr Ala Gly Lys Ala  
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MBI16 Sequence Listing.ST25

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Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys Lys Gly Ala Thr Glu Arg	
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Arg Gly Pro Pro Pro Val Val Tyr Gly Asp Glu Ile Met Glu Glu	
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Lys Pro Lys Val Thr Glu Met Val Met Pro Pro Pro Pro Gln Gln Thr	
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Ser Glu Phe Ala Tyr Phe Asp Thr Ser Asp Ser Val Pro Lys Leu His	
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Thr Thr Asp Ser Ser Cys Ser Glu Gln Val Val Ser Pro Glu Phe Thr	
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Ser Glu Val Gln Ser Glu Pro Lys Trp Lys Asp Trp Ser Ala Val Ser	
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Asn Asp Asn Asn Asn Thr Leu Asp Phe Gly Phe Asn Tyr Ile Asp Ala	
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Thr Val Asp Asn Ala Phe Gly Gly Gly Gly Ser Ser Asn Gln Met Phe	
265 270 275	
ccg cta cag gat atg ttc atg tac atg cag aag cct tac tag	1093
Pro Leu Gln Asp Met Phe Met Tyr Met Gln Lys Pro Tyr	
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35 40 45	

## MBI16 Sequence Listing.ST25

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 85 90 95  
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 100 105 110  
 Ala Gly Lys Ala Pro Lys Gly Glu Lys Thr Asn Trp Ile Met His Glu  
 115 120 125  
 Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys Lys Asn Ser  
 130 135 140  
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 145 150 155 160  
 Ala Thr Glu Arg Arg Gly Pro Pro Pro Pro Val Val Tyr Gly Asp Glu  
 165 170 175  
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 180 185 190  
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 Pro Lys Leu His Thr Thr Asp Ser Ser Cys Ser Glu Gln Val Val Ser  
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 Ser Ala Val Ser Asn Asp Asn Asn Asn Thr Leu Asp Phe Gly Phe Asn  
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## MBI16 Sequence Listing.ST25

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gggtttctga tattgttctt gttctcttga atctttatta cttgaaaaac atataaagtg      180

atg gcg gtt gtg gtt gaa gaa ggt gtg gtg ttg aat cat gga ggt gaa      228
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1      5      10      15

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Glu Leu Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu
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gag atc ata aca tgt tac ctt aag gag aag gtt tta aac agc cga ttc      324
Glu Ile Ile Thr Cys Tyr Leu Lys Glu Lys Val Leu Asn Ser Arg Phe
35      40      45

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Thr Ala Val Ala Met Gly Glu Ala Asp Leu Asn Lys Cys Glu Pro Trp
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gat ttg cca aag agg gca aag atg ggg gag aaa gag ttc tac ttc ttc      420
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65      70      75      80

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Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala
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acg gag tca gga tac tgg aaa gcc acc ggg aag gat aag gag atc ttc      516
Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe
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Lys Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr
115      120      125

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Tyr Arg Leu Glu Gly Lys Tyr Ser Tyr Tyr Asn Leu Pro Lys Ser Ala
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Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Asn Pro Ser
165      170      175

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180      185      190

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Met Asp Ser Leu Glu Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu
195      200      205

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Phe Lys Pro Ile Asn Pro Pro Thr Tyr Asp Ile Ser Ser Pro Ile Gln
225      230      235      240

ccc cat cat ttc aat tct tac caa tca atc ttt aac cac cag gtt ttt      948
Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe
245      250      255

ggt tct gct tcg ggc tct acg tac aac aac aac aac gag atg atc aag      996
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260      265      270

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MBI16 Sequence Listing.ST25

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Val Met Lys Gln Glu Met Gly Met Met Gly Met Val Asn Gly Ser Lys	
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Ser Tyr Glu Asp Leu Cys Asp Leu Arg Gly Asp Leu Trp Asp Phe	
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Thr Ala Val Ala Met Gly Glu Ala Asp Leu Asn Lys Cys Glu Pro Trp	
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Asp Leu Pro Lys Arg Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe	
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Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala	
85 90 95	
Thr Glu Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe	
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Lys Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr	
115 120 125	
Arg Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met His Glu	
130 135 140	
Tyr Arg Leu Glu Gly Lys Tyr Ser Tyr Tyr Asn Leu Pro Lys Ser Ala	
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Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Asn Pro Ser	



## MBI16 Sequence Listing.ST25

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195 200 205

Pro Pro Leu Ile Asp Pro Ser Phe Met Ser Gln Thr Glu Gln Pro Asn  
210 215 220

Phe Lys Pro Ile Asn Pro Pro Thr Tyr Asp Ile Ser Ser Pro Ile Gln  
225 230 235 240

Pro His His Phe Asn Ser Tyr Gln Ser Ile Phe Asn His Gln Val Phe  
245 250 255

Gly Ser Ala Ser Gly Ser Thr Tyr Asn Asn Asn Asn Glu Met Ile Lys  
260 265 270

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275 280 285

Asp Val Asn Ala Asn Met Thr Thr Thr Thr Glu Val Ser Ser Gly Pro  
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MBI16 Sequence Listing.ST25

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## MBI16 Sequence Listing.ST25

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145 150 155 160

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165 170 175

Gly Ser Thr Ser His Val Ser Ser Ser His Arg Gly Phe Asp Leu Asn  
180 185 190

Ile Pro Pro Ile Pro Glu Phe Ser Met Val Asn Gly Asp Asp Glu Val  
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Thr Asn Asn Ser Asp Lys Pro Ser Gln Ala Ala Pro Glu Gln Ser  
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Asn Val His Val Tyr His His Asp Trp Ala Ala Met Gln Ala Tyr Tyr  
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45 50 55  
ggt cat gct cca ccg cct tat atg tgg gcg tct cca tcg cca atg atg 304  
Gly His Ala Pro Pro Pro Tyr Met Trp Ala Ser Pro Ser Pro Met Met  
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MBI16 Sequence Listing.ST25

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cta aac aat gag tct gag aaa cta cgg ctg gag aac gaa gct ata ttg Leu Asn Asn Glu Ser Glu Lys Leu Arg Leu Glu Asn Glu Ala Ile Leu 300 305 310	1024
gat caa ctg aaa gcg caa gca aca ggg aaa aca gag aac ctg atc tct Asp Gln Leu Lys Ala Gln Ala Thr Gly Lys Thr Glu Asn Leu Ile Ser 315 320 325	1072
cga gtt gat aag aac aac tct gta tca ggt agc aaa act gtg cag cat Arg Val Asp Lys Asn Asn Ser Val Ser Gly Ser Lys Thr Val Gln His 330 335 340 345	1120
caa ctg tta aat gca agt ccg ata acc gat cct gtc gcg gct agc tga Gln Leu Leu Asn Ala Ser Pro Ile Thr Asp Pro Val Ala Ala Ser 350 355 360	1168
ccgtggccgc aacaatgaga acccgatatt tcttcctttg gggtgtgatt gtaacttaaa	1228
aggagacttt ttgtttttat tcttagattt gtagctctct gcatagttag cataaattga	1288

## MBI16 Sequence Listing.ST25

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 35 40 45  
 Gln Tyr Tyr Asn Ser Asn Leu Ala Pro Gly His Ala Pro Pro Pro Tyr  
 50 55 60  
 Met Trp Ala Ser Pro Ser Pro Met Met Ala Pro Tyr Gly Ala Pro Tyr  
 65 70 75 80  
 Pro Pro Phe Cys Pro Pro Gly Gly Val Tyr Ala His Pro Gly Val Gln  
 85 90 95  
 Met Gly Ser Gln Pro Gln Gly Pro Val Ser Gln Ser Ala Ser Gly Val  
 100 105 110  
 Thr Thr Pro Leu Thr Ile Asp Ala Pro Ala Asn Ser Ala Gly Asn Ser  
 115 120 125  
 Asp His Gly Phe Met Lys Lys Leu Lys Glu Phe Asp Gly Leu Ala Met  
 130 135 140  
 Ser Ile Ser Asn Asn Lys Val Gly Ser Ala Glu His Ser Ser Ser Glu  
 145 150 155 160  
 His Arg Ser Ser Gln Ser Ser Glu Asn Asp Gly Ser Ser Asn Gly Ser  
 165 170 175  
 Asp Gly Asn Thr Thr Gly Gly Glu Gln Ser Arg Arg Lys Arg Arg Gln  
 180 185 190  
 Gln Arg Ser Pro Ser Thr Gly Glu Arg Pro Ser Ser Gln Asn Ser Leu  
 195 200 205  
 Pro Leu Arg Gly Glu Asn Glu Lys Pro Asp Val Thr Met Gly Thr Pro  
 210 215 220  
 Val Met Pro Thr Ala Met Ser Phe Gln Asn Ser Ala Gly Met Asn Gly  
 225 230 235 240

## MBI16 Sequence Listing.ST25

Val Pro Gln Pro Trp Asn Glu Lys Glu Val Lys Arg Glu Lys Arg Lys  
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Gln Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Leu Arg Lys Gln Ala  
260 265 270

Glu Thr Glu Gln Leu Ser Val Lys Val Asp Ala Leu Val Ala Glu Asn  
275 280 285

Met Ser Leu Arg Ser Lys Leu Gly Gln Leu Asn Asn Glu Ser Glu Lys  
290 295 300

Leu Arg Leu Glu Asn Glu Ala Ile Leu Asp Gln Leu Lys Ala Gln Ala  
305 310 315 320

Thr Gly Lys Thr Glu Asn Leu Ile Ser Arg Val Asp Lys Asn Asn Ser  
325 330 335

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Ile Thr Asp Pro Val Ala Ala Ser  
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Met Gly Arg Ser  
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ccg tgc tgt gag aaa gct cac aca aac aaa gga gca tgg acg aaa gaa 163  
Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala Trp Thr Lys Glu  
5 10 15 20  
gag gac gag agg ctc gtc gcc tac att aaa gct cat gga gaa ggc tgc 211  
Glu Asp Glu Arg Leu Val Ala Tyr Ile Lys Ala His Gly Glu Gly Cys  
25 30 35  
tgg aga tct ctc ccc aaa gcc gcc gga ctt ctt cgc tgt ggc aag agc 259  
Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser  
40 45 50  
tgc cgt ctc cgg tgg atc aac tat ctc cgg cct gac ctt aag cgt gga 307  
Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly  
55 60 65  
aac ttc acc gag gaa gaa gac gaa ctc atc atc aag ctc cat agc ctt 355  
Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu  
70 75 80  
ctt ggc aac aaa tgg tgg ctt att gcc ggg aga tta ccg gga aga aca 403  
Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu Pro Gly Arg Thr  
85 90 95 100  
gat aac gag ata aag aac tat tgg aac acg cat ata cga aga aag ctt 451

MBI16 Sequence Listing.ST25

Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile Arg Arg Lys Leu	
105 110 115	
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Ile Asn Arg Gly Ile Asp Pro Thr Ser His Arg Pro Ile Gln Glu Ser	
120 125 130	
tca gct tct caa gat tct aaa cct aca caa cta gaa cca gtt acg agt	547
Ser Ala Ser Gln Asp Ser Lys Pro Thr Gln Leu Glu Pro Val Thr Ser	
135 140 145	
aat acc att aat atc tca ttc act tct gct cca aag gtc gaa acg ttc	595
Asn Thr Ile Asn Ile Ser Phe Pro Thr Ser Ala Pro Lys Val Glu Thr Phe	
150 155 160	
cat gaa agt ata agc ttt ccg gga aaa tca gag aaa atc tca atg ctt	643
His Glu Ser Ile Ser Phe Pro Gly Lys Ser Glu Lys Ile Ser Met Leu	
165 170 175 180	
acg ttc aaa gaa gaa aaa gat gag tgc cca gtt caa gaa aag ttc cca	691
Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gln Glu Lys Phe Pro	
185 190 195	
gat ttg aat ctt gag ctc aga atc agt ctt cct gat gat gtt gat cgt	739
Asp Leu Asn Leu Glu Leu Arg Ile Ser Leu Pro Asp Asp Val Asp Arg	
200 205 210	
ctt caa ggg cat gga aag tca aca acg cca cgt tgt ttc aag tgc agc	787
Leu Gln Gly His Gly Lys Ser Thr Thr Pro Arg Cys Phe Lys Cys Ser	
215 220 225	
tta ggg atg ata aac ggc atg gag tgc aga tgc gga aga atg aga tgc	835
Leu Gly Met Ile Asn Gly Met Glu Cys Arg Cys Gly Arg Met Arg Cys	
230 235 240	
gat gta gtc gga ggt agc agc aag ggg agt gac atg agc aat gga ttt	883
Asp Val Val Gly Gly Ser Ser Lys Gly Ser Asp Met Ser Asn Gly Phe	
245 250 255 260	
gat ttt tta ggg ttg gca aag aaa gag acc act tct ctt ttg ggc ttt	931
Asp Phe Leu Gly Leu Ala Lys Lys Glu Thr Thr Ser Leu Leu Gly Phe	
265 270 275	
cga agc ttg gag atg aaa taa tattgtcaaa ttttaggcgt aactgtacaa	982
Arg Ser Leu Glu Met Lys	
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Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg	
35 40 45	
Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp	
50 55 60	

## MBI16 Sequence Listing.ST25

Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu Ile Ile Lys  
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 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Gly Arg Leu  
 85 90 95  
 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile  
 100 105 110  
 Arg Arg Lys Leu Ile Asn Arg Gly Ile Asp Pro Thr Ser His Arg Pro  
 115 120 125  
 Ile Gln Glu Ser Ser Ala Ser Gln Asp Ser Lys Pro Thr Gln Leu Glu  
 130 135 140  
 Pro Val Thr Ser Asn Thr Ile Asn Ile Ser Phe Thr Ser Ala Pro Lys  
 145 150 155 160  
 Val Glu Thr Phe His Glu Ser Ile Ser Phe Pro Gly Lys Ser Glu Lys  
 165 170 175  
 Ile Ser Met Leu Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gln  
 180 185 190  
 Glu Lys Phe Pro Asp Leu Asn Leu Glu Leu Arg Ile Ser Leu Pro Asp  
 195 200 205  
 Asp Val Asp Arg Leu Gln Gly His Gly Lys Ser Thr Thr Pro Arg Cys  
 210 215 220  
 Phe Lys Cys Ser Leu Gly Met Ile Asn Gly Met Glu Cys Arg Cys Gly  
 225 230 235 240  
 Arg Met Arg Cys Asp Val Val Gly Gly Ser Ser Lys Gly Ser Asp Met  
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 act tct tct tct gaa gaa gtg agt agt ctt gag tgg gaa gtt gtg aac 96  
 Thr Ser Ser Ser Glu Glu Val Ser Ser Leu Glu Trp Glu Val Val Asn  
 20 25 30



## MBI16 Sequence Listing.ST25

atg agt caa gaa gaa gaa gat ttg gtc tct cga atg cat aag ctt gtc 144  
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35 40 45

ggc gac agg tgg gaa ctg ata gct ggg agg atc cca gga aga acc gct 192  
Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala  
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gga gaa att gag agg ttt tgg gtc atg aaa aat tga 228  
Gly Glu Ile Glu Arg Phe Trp Val Met Lys Asn  
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<212> PRT  
<213> Arabidopsis thaliana

<400> 28

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Thr Ser Ser Ser Glu Glu Val Ser Ser Leu Glu Trp Glu Val Val Asn  
20 25 30

Met Ser Gln Glu Glu Glu Asp Leu Val Ser Arg Met His Lys Leu Val  
35 40 45

Gly Asp Arg Trp Glu Leu Ile Ala Gly Arg Ile Pro Gly Arg Thr Ala  
50 55 60

Gly Glu Ile Glu Arg Phe Trp Val Met Lys Asn  
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ata tta aag ata ctt tac gtc atc ggt ttc ttt aga gac atg gtc gat 96  
Ile Leu Lys Ile Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp  
20 25 30

gct ctt tgt cct tac att ggt cta cct agt ttt cta gac cac aac gag 144  
Ala Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu  
35 40 45

acc tct gga ccc gat ccg acc cga cac gct ctg tct acg tca gcg agt 192  
Thr Ser Gly Pro Asp Pro Thr Arg His Ala Leu Ser Thr Ser Ala Ser  
50 55 60

ctt gct aac gag ttg atc ccg gtg gtt cgg ttc tcg gat ctt ccg acc 240  
Leu Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Pro Thr  
65 70 75 80

gat ccg gaa gat tgt tgt acg gtt tgt ttg tca gat ttt gag tcc gac 288  
Asp Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Glu Ser Asp

## MBI16 Sequence Listing.ST25

85	90	95	
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Asp Lys Val Arg Gln Leu Pro Lys Cys Gly His Val Phe His His His	100	105 110	
tgt tta gac cgt tgg atc gtt gac tac aac aag atg aaa tgt ccg gtt			384
Cys Leu Asp Arg Trp Ile Val Asp Tyr Asn Lys Met Lys Cys Pro Val	115	120 125	
tgt cgg cac cgg ttc tta ccg aaa gaa aag tac acg caa tgt gat tgg			432
Cys Arg His Arg Phe Leu Pro Lys Glu Lys Tyr Thr Gln Cys Asp Trp	130	135 140	
ggg tct ggt tca gat tgg ttt agt gat gaa gtg gaa agt acc aac taa			480
Gly Ser Gly Ser Asp Trp Phe Ser Asp Glu Val Glu Ser Thr Asn	145	150 155	

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Ala Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu	35	40	45	
Thr Ser Gly Pro Asp Pro Thr Arg His Ala Leu Ser Thr Ser Ala Ser	50	55	60	
Leu Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Pro Thr	65	70	75	80
Asp Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Glu Ser Asp	85	90	95	
Asp Lys Val Arg Gln Leu Pro Lys Cys Gly His Val Phe His His His	100	105	110	
Cys Leu Asp Arg Trp Ile Val Asp Tyr Asn Lys Met Lys Cys Pro Val	115	120	125	
Cys Arg His Arg Phe Leu Pro Lys Glu Lys Tyr Thr Gln Cys Asp Trp	130	135	140	
Gly Ser Gly Ser Asp Trp Phe Ser Asp Glu Val Glu Ser Thr Asn	145	150	155	

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ttcctgaaac tgttgagttc ttgtgaaagg aaataaaaaa c atg atg atg ggc aaa      176
                                         Met Met Met Gly Lys
                                         1 5
gaa gat cta ggt ttg agc cta agc tta ggg ttt tca caa aat cac aat      224
Glu Asp Leu Gly Leu Ser Leu Ser Leu Gly Phe Ser Gln Asn His Asn
                                         10 15 20
cct ctt cag atg aat ctg aat cct aac tct tca tta tca aac aat ctc      272
Pro Leu Gln Met Asn Leu Asn Pro Asn Ser Ser Leu Ser Asn Asn Leu
                                         25 30 35
cag aga ctc cca tgg aac caa aca ttc gat cct aca tca gat ctt cgc      320
Gln Arg Leu Pro Trp Asn Gln Thr Phe Asp Pro Thr Ser Asp Leu Arg
                                         40 45 50
aag ata gac gtg aac agt ttt cca tca acg gtt aac tgc gag gaa gac      368
Lys Ile Asp Val Asn Ser Phe Pro Ser Thr Val Asn Cys Glu Glu Asp
                                         55 60 65
aca gga gtt tcg tca cca aac agt acg atc tca agc acc att agc ggg      416
Thr Gly Val Ser Ser Pro Asn Ser Thr Ile Ser Ser Thr Ile Ser Gly
                                         70 75 80 85
aag aga agt gag aga gaa gga atc tcc gga acc ggc gtt ggc tcc ggc      464
Lys Arg Ser Glu Arg Glu Gly Ile Ser Gly Thr Gly Val Gly Ser Gly
                                         90 95 100
gac gat cac gac gag atc act ccg gat cga ggg tac tca cgt gga acc      512
Asp Asp His Asp Glu Ile Thr Pro Asp Arg Gly Tyr Ser Arg Gly Thr
                                         105 110 115
tca gat gaa gaa gaa gac ggg ggc gaa acg tcg agg aag aag ctc agg      560
Ser Asp Glu Glu Glu Asp Gly Gly Glu Thr Ser Arg Lys Lys Leu Arg
                                         120 125 130
tta tca aaa gat cag tct gct ttt ctc gaa gag act ttc aaa gaa cac      608
Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Glu Thr Phe Lys Glu His
                                         135 140 145
aac act ctc aat ccc aaa cag aag cta gct ttg gct aag aag ctg aac      656
Asn Thr Leu Asn Pro Lys Gln Lys Leu Ala Leu Ala Lys Lys Leu Asn
                                         150 155 160 165
ttg acg gca aga caa gtg gaa gtg tgg ttc caa aac aga aga gct aga      704
Leu Thr Ala Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala Arg
                                         170 175 180
acc aag tta aag caa acg gag gta gat tgc gaa tac ttg aaa cgg tgc      752
Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys Arg Cys
                                         185 190 195
gta gag aag cta acg gaa gag aac cgg aga ctt cag aaa gag gct atg      800
Val Glu Lys Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu Ala Met
                                         200 205 210
gag ctt cga act ctc aag ctg tct cca caa ttc tac ggt cag atg act      848
Glu Leu Arg Thr Leu Lys Leu Ser Pro Gln Phe Tyr Gly Gln Met Thr
                                         215 220 225
cca cca act aca ctc atc atg tgt cct tcg tgc gag cgt gta gct ggt      896
Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys Glu Arg Val Ala Gly
                                         230 235 240 245
cca tca tca tcg aac cat cac cac aat cac agg ccg gtt tcg att aac      944
Pro Ser Ser Ser Asn His His His Asn His Arg Pro Val Ser Ile Asn
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MBI16 Sequence Listing.ST25

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      265                      270                      275

gcc ttg cgt cca cga tcg taa ttttttagtgg tgggggaagg gtgttttggg    1043
Ala Leu Arg Pro Arg Ser
      280

ttttttcatt atcgttatat agtctatctg tgtgggggtca ttgtaatttt ggatgattgg    1103

ccttctcatg aactagtcac atgtatgatg caaccttaaa aatattttcaa gtagcaaaac    1163

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<211> 283
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Leu Ser Asn Asn Leu Gln Arg Leu Pro Trp Asn Gln Thr Phe Asp Pro
      35      40      45

Thr Ser Asp Leu Arg Lys Ile Asp Val Asn Ser Phe Pro Ser Thr Val
      50      55      60

Asn Cys Glu Glu Asp Thr Gly Val Ser Ser Pro Asn Ser Thr Ile Ser
      65      70      75      80

Ser Thr Ile Ser Gly Lys Arg Ser Glu Arg Glu Gly Ile Ser Gly Thr
      85      90      95

Gly Val Gly Ser Gly Asp Asp His Asp Glu Ile Thr Pro Asp Arg Gly
      100      105      110

Tyr Ser Arg Gly Thr Ser Asp Glu Glu Glu Asp Gly Gly Glu Thr Ser
      115      120      125

Arg Lys Lys Leu Arg Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Glu
      130      135      140

Thr Phe Lys Glu His Asn Thr Leu Asn Pro Lys Gln Lys Leu Ala Leu
      145      150      155      160

Ala Lys Lys Leu Asn Leu Thr Ala Arg Gln Val Glu Val Trp Phe Gln
      165      170      175

Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu
      180      185      190

Tyr Leu Lys Arg Cys Val Glu Lys Leu Thr Glu Glu Asn Arg Arg Leu
      195      200      205

Gln Lys Glu Ala Met Glu Leu Arg Thr Leu Lys Leu Ser Pro Gln Phe

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## MBI16 Sequence Listing.ST25

210 215 220

Tyr Gly Gln Met Thr Pro Pro Thr Thr Leu Ile Met Cys Pro Ser Cys  
225 230 235 240

Glu Arg Val Ala Gly Pro Ser Ser Ser Asn His His His Asn His Arg  
245 250 255

Pro Val Ser Ile Asn Pro Trp Ile Ala Cys Ala Gly Gln Val Ala His  
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Met Gly Leu Asp Asp Ser Cys Asn Thr Gly  
1 5 10

ctt gtt ctt ggt tta ggc ctc tca cca acg cct aat aat tac aat cat 159  
Leu Val Leu Gly Leu Gly Leu Ser Pro Thr Pro Asn Asn Tyr Asn His  
15 20 25

gcc atc aag aaa tct tcc tcc act gtg gac cat cgt ttc atc agg ctc 207  
Ala Ile Lys Lys Ser Ser Ser Thr Val Asp His Arg Phe Ile Arg Leu  
30 35 40

gat ccg tcg ttg act cta agc cta tcc ggt gag agc tac aag atc aag 255  
Asp Pro Ser Leu Thr Leu Ser Leu Ser Gly Glu Ser Tyr Lys Ile Lys  
45 50 55

act ggt gcc ggc gcc ggc gac caa att tgc cgg cag acc tgc tcc cac 303  
Thr Gly Ala Gly Ala Gly Asp Gln Ile Cys Arg Gln Thr Ser Ser His  
60 65 70

agc ggc atc tca tct ttc tcg agc gga agg gta aag aga gaa aga gaa 351  
Ser Gly Ile Ser Ser Phe Ser Ser Gly Arg Val Lys Arg Glu Arg Glu  
75 80 85 90

atc tcc ggc ggc gat gga gaa gaa gag gcg gag gag acg acg gag aga 399  
Ile Ser Gly Gly Asp Gly Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg  
95 100 105

gtg gtg tgt tcg aga gtg agt gat gat cat gac gat gaa gaa ggt gtt 447  
Val Val Cys Ser Arg Val Ser Asp Asp His Asp Asp Glu Glu Gly Val  
110 115 120

agt gct cgt aaa aag ctt aga ctc act aaa caa caa tct gct ctt ctc 495  
Ser Ala Arg Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu  
125 130 135

gaa gat aac ttc aaa ctt cat agc acc ctt aat ccc aag caa aaa caa 543  
Glu Asp Asn Phe Lys Leu His Ser Thr Leu Asn Pro Lys Gln Lys Gln  
140 145 150

gct ctt gcg aga cag ctg aat cta agg cct aga caa gtt gaa gtg tgg 591  
Ala Leu Ala Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp

MBI16 Sequence Listing.ST25

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Phe Gln Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp	175	180	185	
tgt gag ttt ttg aag aaa tgt tgc gag act tta acg gat gag aat aga				687
Cys Glu Phe Leu Lys Lys Cys Cys Glu Thr Leu Thr Asp Glu Asn Arg	190	195	200	
agg ctt caa aaa gag ctt caa gac ctt aag gct tta aaa ttg tct caa				735
Arg Leu Gln Lys Glu Leu Gln Asp Leu Lys Ala Leu Lys Leu Ser Gln	205	210	215	
ccg ttt tac atg cac atg ccg gcg gcg act ttg act atg tgc cct tct				783
Pro Phe Tyr Met His Met Pro Ala Ala Thr Leu Thr Met Cys Pro Ser	220	225	230	
tgt gag aga ctc ggc ggt ggt ggt gtc gga gga gat acg acg gcg gtt				831
Cys Glu Arg Leu Gly Gly Gly Gly Val Gly Gly Asp Thr Thr Ala Val	235	240	245	250
gat gaa gaa acg gcg aaa gga gct ttc tcc atc gtc aca aag cct cgt				879
Asp Glu Glu Thr Ala Lys Gly Ala Phe Ser Ile Val Thr Lys Pro Arg	255	260	265	
ttc tat aac cct ttc act aat cct tct gca gca tgt tag ttacttatta				928
Phe Tyr Asn Pro Phe Thr Asn Pro Ser Ala Ala Cys	270	275		
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agaagataaa tcccagggaa aaaatattac gttgaaattg gggggaaatg gggatatagtc				1048
tttatagata agactcttca acgattccac tttatttttc ggtgggattg ttggttgatg				1108
aagaaaaaaa aatagtttgt aattacaggt ttaaatatgt agagaaaaaa tgacgaatat				1168
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 <212> PRT  
 <213> Arabidopsis thaliana

<400> 34

Met Gly Leu Asp Asp Ser Cys Asn Thr Gly Leu Val Leu Gly Leu Gly  
 1 5 10 15

Leu Ser Pro Thr Pro Asn Asn Tyr Asn His Ala Ile Lys Lys Ser Ser  
 20 25 30

Ser Thr Val Asp His Arg Phe Ile Arg Leu Asp Pro Ser Leu Thr Leu  
 35 40 45

Ser Leu Ser Gly Glu Ser Tyr Lys Ile Lys Thr Gly Ala Gly Ala Gly  
 50 55 60

Asp Gln Ile Cys Arg Gln Thr Ser Ser His Ser Gly Ile Ser Ser Phe  
 65 70 75 80

Ser Ser Gly Arg Val Lys Arg Glu Arg Glu Ile Ser Gly Gly Asp Gly  
 85 90 95

Glu Glu Glu Ala Glu Glu Thr Thr Glu Arg Val Val Cys Ser Arg Val

MB116 Sequence Listing.ST25

100	105	110	
Ser Asp Asp His Asp Asp Glu Glu Gly Val Ser Ala Arg Lys Lys Leu			
115	120	125	
Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu Glu Asp Asn Phe Lys Leu			
130	135	140	
His Ser Thr Leu Asn Pro Lys Gln Lys Gln Ala Leu Ala Arg Gln Leu			
145	150	155	160
Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg Ala			
	165	170	175
Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe Leu Lys Lys			
	180	185	190
Cys Cys Glu Thr Leu Thr Asp Glu Asn Arg Arg Leu Gln Lys Glu Leu			
	195	200	205
Gln Asp Leu Lys Ala Leu Lys Leu Ser Gln Pro Phe Tyr Met His Met			
210	215	220	
Pro Ala Ala Thr Leu Thr Met Cys Pro Ser Cys Glu Arg Leu Gly Gly			
225	230	235	240
Gly Gly Val Gly Gly Asp Thr Thr Ala Val Asp Glu Glu Thr Ala Lys			
	245	250	255
Gly Ala Phe Ser Ile Val Thr Lys Pro Arg Phe Tyr Asn Pro Phe Thr			
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Asn Pro Ser Ala Ala Cys			
275			

<210> 35  
 <211> 1147  
 <212> DNA  
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<220>  
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 <222> (33)..(695)  
 <223> G489

<400> 35	
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Met Asn Tyr Gly Thr Asn Pro	
1 5	
tac caa acc aac ccg atg agc acc act gct gct act gta gca gga ggt	101
Tyr Gln Thr Asn Pro Met Ser Thr Thr Ala Ala Thr Val Ala Gly Gly	
10 15 20	
gcg gca caa cca ggc cag ctg gcg ttc cac cag atc cat cag cag cag	149
Ala Ala Gln Pro Gly Gln Leu Ala Phe His Gln Ile His Gln Gln Gln	
25 30 35	
cag cag caa cag ctg gca cag cag ctt caa gca ttt tgg gag aac caa	197
Gln Gln Gln Gln Leu Ala Gln Gln Leu Gln Ala Phe Trp Glu Asn Gln	
40 45 50 55	

MBI16 Sequence Listing.ST25

ttc aaa gag att gag aag act acc gat ttc aag aac cac agc ctt ccc Phe Lys Glu Ile Glu Lys Thr Thr Asp Phe Lys Asn His Ser Leu Pro 60 65 70	245
ctt gcg aga atc aag aaa atc atg aaa gcg gat gaa gat gtc cgt atg Leu Ala Arg Ile Lys Lys Ile Met Lys Ala Asp Glu Asp Val Arg Met 75 80 85	293
atc tcg gct gag gcg ccg gtc gtg ttt gca agg gcc tgt gag atg ttc Ile Ser Ala Glu Ala Pro Val Val Phe Ala Arg Ala Cys Glu Met Phe 90 95 100	341
atc ctg gag ctg aca ctc agg tcg tgg aac cac act gag gag aat aag Ile Leu Glu Leu Thr Leu Arg Ser Trp Asn His Thr Glu Glu Asn Lys 105 110 115	389
agg cgg acg ttg cag aag aac gat att gct gct gct gtg act aga acc Arg Arg Thr Leu Gln Lys Asn Asp Ile Ala Ala Ala Val Thr Arg Thr 120 125 130 135	437
gat att ttt gat ttc ctt gtg gat att gtt ccc cgg gag gat ctc cga Asp Ile Phe Asp Phe Leu Val Asp Ile Val Pro Arg Glu Asp Leu Arg 140 145 150	485
gat gaa gtc ttg gga agt att ccg agg ggc act gtc ccg gaa gct gct Asp Glu Val Leu Gly Ser Ile Pro Arg Gly Thr Val Pro Glu Ala Ala 155 160 165	533
gct gct ggt tac ccg tat gga tac ttg cct gca gga act gct cca ata Ala Ala Gly Tyr Pro Tyr Gly Tyr Leu Pro Ala Gly Thr Ala Pro Ile 170 175 180	581
gga aat ccg gga atg gtt atg ggt aat ccc ggt ggt gcg tat cca cct Gly Asn Pro Gly Met Val Met Gly Asn Pro Gly Gly Ala Tyr Pro Pro 185 190 195	629
aat cct tat atg ggt caa cca atg tgg caa caa cag gca cct gac caa Asn Pro Tyr Met Gly Gln Pro Met Trp Gln Gln Gln Ala Pro Asp Gln 200 205 210 215	677
cct gac cag gaa aat tag caagaaactg tgagtcttcc agcttcgcgg Pro Asp Gln Glu Asn 220	725
ccgctctaga caggcctcgt accggatcct ctagctagag ctttcgttcg tatcatcggt	785
ttcgacaacg ttcgtcaagt tcaatgcata agtttcattg cgcacacacc agaatacctac	845
tgagtttgag tattatggca ttgggaaaac tgtttttctt gtccatttgt tgtgcttgta	905
atctactgtg ttttttatcc ggttttcgct atcgaaactgt gaaatggaaa tggatggaga	965
agagttaatg aatgatatgg ccttttgttc attctcaaat taatattatt tggtttttct	1025
cttatttgtg gggatgaatt tgaaattata agagatatgc aaacattttg tttgagtaaa	1085
atgtgcaaat cgtggcctct aatgacccga agttaatatg aggagtaaaa cacttgtagg	1145
tg	1147

<210> 36  
 <211> 220  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 36

Met Asn Tyr Gly Thr Asn Pro Tyr Gln Thr Asn Pro Met Ser Thr Thr  
 1 5 10 15

Ala Ala Thr Val Ala Gly Gly Ala Ala Gln Pro Gly Gln Leu Ala Phe  
 20 25 30



## MBI16 Sequence Listing.ST25

His Gln Ile His Gln Gln Gln Gln Gln Gln Gln Leu Ala Gln Gln Leu  
35 40 45

Gln Ala Phe Trp Glu Asn Gln Phe Lys Glu Ile Glu Lys Thr Thr Asp  
50 55 60

Phe Lys Asn His Ser Leu Pro Leu Ala Arg Ile Lys Lys Ile Met Lys  
65 70 75 80

Ala Asp Glu Asp Val Arg Met Ile Ser Ala Glu Ala Pro Val Val Phe  
85 90 95

Ala Arg Ala Cys Glu Met Phe Ile Leu Glu Leu Thr Leu Arg Ser Trp  
100 105 110

Asn His Thr Glu Glu Asn Lys Arg Arg Thr Leu Gln Lys Asn Asp Ile  
115 120 125

Ala Ala Ala Val Thr Arg Thr Asp Ile Phe Asp Phe Leu Val Asp Ile  
130 135 140

Val Pro Arg Glu Asp Leu Arg Asp Glu Val Leu Gly Ser Ile Pro Arg  
145 150 155 160

Gly Thr Val Pro Glu Ala Ala Ala Ala Gly Tyr Pro Tyr Gly Tyr Leu  
165 170 175

Pro Ala Gly Thr Ala Pro Ile Gly Asn Pro Gly Met Val Met Gly Asn  
180 185 190

Pro Gly Gly Ala Tyr Pro Pro Asn Pro Tyr Met Gly Gln Pro Met Trp  
195 200 205

Gln Gln Gln Ala Pro Asp Gln Pro Asp Gln Glu Asn  
210 215 220

<210> 37  
<211> 1262  
<212> DNA  
<213> Arabidopsis thaliana

<220>  
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<222> (217)..(957)  
<223> G463

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ctttcaaaag actctttcct tcttttggat tgattttgga ttctagggct ctctttcttt 180  
tagtggtgtt ttgttgttgt tgttgtgggc tctctg atg att act gaa ctt gag 234  
Met Ile Thr Glu Leu Glu  
1 5  
atg ggg aaa ggt gag agt gag ctt gag ctt ggt cta ggg ctg agt ctt 282  
Met Gly Lys Gly Glu Ser Glu Leu Glu Leu Gly Leu Gly Leu Ser Leu  
10 15 20

MBI16 Sequence Listing.ST25

ggc ggt gga acg gcg gcc aag att ggt aaa tca ggt ggt ggt ggc gcg Gly Gly Gly Thr Ala Ala Lys Ile Gly Lys Ser Gly Gly Gly Gly Ala 25 30 35	330
tgg gga gag cgt gga agg ctt ttg acg gct aag gat ttt cct tct gtt Trp Gly Glu Arg Gly Arg Leu Leu Thr Ala Lys Asp Phe Pro Ser Val 40 45 50	378
ggt tct aaa cgt gct gct gat tct gct tct cat gct ggt tca tct cct Gly Ser Lys Arg Ala Ala Asp Ser Ala Ser His Ala Gly Ser Ser Pro 55 60 65 70	426
cct cgt tca agt caa gtt gtt gga tgg cct cct ata ggg tca cac agg Pro Arg Ser Ser Gln Val Val Gly Trp Pro Pro Ile Gly Ser His Arg 75 80 85	474
atg aac agt ttg gtt aat aac caa gct aca aag tca gca aga gaa gaa Met Asn Ser Leu Val Asn Asn Gln Ala Thr Lys Ser Ala Arg Glu Glu 90 95 100	522
gaa gaa gct ggt aag aag aaa gtg aaa gat gat gaa cct aaa gat gtg Glu Glu Ala Gly Lys Lys Lys Val Lys Asp Asp Glu Pro Lys Asp Val 105 110 115	570
aca aag aaa gtg aat ggg aaa gta caa gtt gga ttt att aag gtg aac Thr Lys Lys Val Asn Gly Lys Val Gln Val Gly Phe Ile Lys Val Asn 120 125 130	618
atg gat gga gtt gct ata gga aga aaa gtg gat ttg aat gct cat tct Met Asp Gly Val Ala Ile Gly Arg Lys Val Asp Leu Asn Ala His Ser 135 140 145 150	666
tct tac gag aat ttg gcg caa aca ttg gaa gat atg ttc ttt cgc act Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu Asp Met Phe Phe Arg Thr 155 160 165	714
aat ccg ggt act gtc ggg tta acc agt cag ttc act aaa ccg ttg agg Asn Pro Gly Thr Val Gly Leu Thr Ser Gln Phe Thr Lys Pro Leu Arg 170 175 180	762
ctt tta gat gga tcg tct gag ttt gta ctt act tat gaa gat aag gaa Leu Leu Asp Gly Ser Ser Glu Phe Val Leu Thr Tyr Glu Asp Lys Glu 185 190 195	810
gga gat tgg atg ctt gtt ggt gat gtt cca tgg aga atg ttc atc aac Gly Asp Trp Met Leu Val Gly Asp Val Pro Trp Arg Met Phe Ile Asn 200 205 210	858
tcg gtg aaa agg cta cgt gtg atg aaa acc tct gaa gct aat gga ctc Ser Val Lys Arg Leu Arg Val Met Lys Thr Ser Glu Ala Asn Gly Leu 215 220 225 230	906
gct gca cga aat caa gaa cca aac gag aga cag cga aag cag ccg gtt Ala Ala Arg Asn Gln Glu Pro Asn Glu Arg Gln Arg Lys Gln Pro Val 235 240 245	954
tag atctcttttc gacgttacgg tggtacaggt tttatatattt ggggttttgc	1007
aagtctgaga tacttctgaa gcaagcataa gctagattga tcttatatcc agtttggtga	1067
ttttcttggt tcttataatg gtttttactg gttttcttta gttttttttt ttgctgtctt	1127
ttaatttttcg gttgcgattt cactatatac tatggatgga agagaatgct ctttatatct	1187
tttactacac tgtaaattatt tgaagcttat ctaatatcgt ttttaagggt taaaaaaccc	1247
tgacgtagcc tcgag	1262
<210> 38	
<211> 246	
<212> PRT	
<213> Arabidopsis thaliana	
<400> 38	

## MBI16 Sequence Listing.ST25

Met Ile Thr Glu Leu Glu Met Gly Lys Gly Glu Ser Glu Leu Glu Leu  
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 Gly Leu Gly Leu Ser Leu Gly Gly Gly Thr Ala Ala Lys Ile Gly Lys  
 20 25 30  
 Ser Gly Gly Gly Gly Ala Trp Gly Glu Arg Gly Arg Leu Leu Thr Ala  
 35 40 45  
 Lys Asp Phe Pro Ser Val Gly Ser Lys Arg Ala Ala Asp Ser Ala Ser  
 50 55 60  
 His Ala Gly Ser Ser Pro Pro Arg Ser Ser Gln Val Val Gly Trp Pro  
 65 70 75 80  
 Pro Ile Gly Ser His Arg Met Asn Ser Leu Val Asn Asn Gln Ala Thr  
 85 90 95  
 Lys Ser Ala Arg Glu Glu Glu Glu Ala Gly Lys Lys Lys Val Lys Asp  
 100 105 110  
 Asp Glu Pro Lys Asp Val Thr Lys Lys Val Asn Gly Lys Val Gln Val  
 115 120 125  
 Gly Phe Ile Lys Val Asn Met Asp Gly Val Ala Ile Gly Arg Lys Val  
 130 135 140  
 Asp Leu Asn Ala His Ser Ser Tyr Glu Asn Leu Ala Gln Thr Leu Glu  
 145 150 155 160  
 Asp Met Phe Phe Arg Thr Asn Pro Gly Thr Val Gly Leu Thr Ser Gln  
 165 170 175  
 Phe Thr Lys Pro Leu Arg Leu Leu Asp Gly Ser Ser Glu Phe Val Leu  
 180 185 190  
 Thr Tyr Glu Asp Lys Glu Gly Asp Trp Met Leu Val Gly Asp Val Pro  
 195 200 205  
 Trp Arg Met Phe Ile Asn Ser Val Lys Arg Leu Arg Val Met Lys Thr  
 210 215 220  
 Ser Glu Ala Asn Gly Leu Ala Ala Arg Asn Gln Glu Pro Asn Glu Arg  
 225 230 235 240  
 Gln Arg Lys Gln Pro Val  
 245

<210> 39  
 <211> 905  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <221> CDS  
 <222> (76)..(837)  
 <223> G767

## MBI16 Sequence Listing.ST25

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gaaaataggt taatt atg atg aaa tct ggg gct gat ttg caa ttt cca cca      111
      Met Met Lys Ser Gly Ala Asp Leu Gln Phe Pro Pro
      1          5          10

gga ttt aga ttt cat cct acg gat gag gag cta gtc ctc atg tat ctc      159
Gly Phe Arg Phe His Pro Thr Asp Glu Glu Leu Val Leu Met Tyr Leu
      15          20          25

tgt cgt aaa tgc gcg tcg cag ccg atc cct gct ccg att atc acc gaa      207
Cys Arg Lys Cys Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu
      30          35          40

ctc gat ttg tac cga tat gat cct tgg gac ctt ccc gac atg gct ttg      255
Leu Asp Leu Tyr Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu
      45          50          55

tac ggt gaa aag gag tgg tat ttt ttc tca cca aga gat cga aag tat      303
Tyr Gly Glu Lys Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr
      65          70          75

cca aac ggt tca aga ccc aac cgt gca gct ggt act gga tat tgg aaa      351
Pro Asn Gly Ser Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys
      80          85          90

gct acc gga gct gat aaa cca ata ggt cgt cct aaa ccg gtt ggt att      399
Ala Thr Gly Ala Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile
      95          100          105

aag aag gct cta gtg ttt tac tcg gga aaa cct cca aat gga gag aaa      447
Lys Lys Ala Leu Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys
      110          115          120

acc aat tgg att atg cac gaa tac cgg ctc gct gac gtt gac cgg tcg      495
Thr Asn Trp Ile Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser
      125          130          135          140

gtt cgt aag aaa aac agt cta aga ttg gac gat tgg gta ttg tgt cgt      543
Val Arg Lys Lys Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg
      145          150          155

ata tat aac aag aaa ggt gtc atc gag aag cga cga agc gat atc gag      591
Ile Tyr Asn Lys Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu
      160          165          170

gac ggg tta aag cct gtg act gac acg tgt cca ccg gaa tct gtg gcg      639
Asp Gly Leu Lys Pro Val Thr Asp Thr Cys Pro Pro Glu Ser Val Ala
      175          180          185

aga ttg atc tcc ggc tcg gag caa gcg gtg tca ccg gaa ttc acg tgt      687
Arg Leu Ile Ser Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys
      190          195          200

agc aac ggt cgg ttg agt aat gcc ctt gat ttt ccg ttt aat tac gta      735
Ser Asn Gly Arg Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val
      205          210          215          220

gat gcc atc gcc gat aac gag att gtg tca cgg cta ttg ggc ggc aat      783
Asp Ala Ile Ala Asp Asn Glu Ile Val Ser Arg Leu Leu Gly Gly Asn
      225          230          235

cag atg tgg tcg acg acg ctt gat cca ctt gtg gtt agg cag gga act      831
Gln Met Trp Ser Thr Thr Leu Asp Pro Leu Val Val Arg Gln Gly Thr
      240          245          250

ttc tga gttgtcacgt gcgattagag ttagtgga aa gtggaacta tcactgtctg      887
Phe

ttttcgcacg tgtcgggc      905

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## MBI16 Sequence Listing.ST25

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<210> 40
<211> 253
<212> PRT
<213> Arabidopsis thaliana

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Met Met Lys Ser Gly Ala Asp Leu Gln Phe Pro Pro Gly Phe Arg Phe
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His Pro Thr Asp Glu Glu Leu Val Leu Met Tyr Leu Cys Arg Lys Cys
20      25      30

Ala Ser Gln Pro Ile Pro Ala Pro Ile Ile Thr Glu Leu Asp Leu Tyr
35      40      45

Arg Tyr Asp Pro Trp Asp Leu Pro Asp Met Ala Leu Tyr Gly Glu Lys
50      55      60

Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr Pro Asn Gly Ser
65      70      75      80

Arg Pro Asn Arg Ala Ala Gly Thr Gly Tyr Trp Lys Ala Thr Gly Ala
85      90      95

Asp Lys Pro Ile Gly Arg Pro Lys Pro Val Gly Ile Lys Lys Ala Leu
100     105     110

Val Phe Tyr Ser Gly Lys Pro Pro Asn Gly Glu Lys Thr Asn Trp Ile
115     120     125

Met His Glu Tyr Arg Leu Ala Asp Val Asp Arg Ser Val Arg Lys Lys
130     135     140

Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg Ile Tyr Asn Lys
145     150     155     160

Lys Gly Val Ile Glu Lys Arg Arg Ser Asp Ile Glu Asp Gly Leu Lys
165     170     175

Pro Val Thr Asp Thr Cys Pro Pro Glu Ser Val Ala Arg Leu Ile Ser
180     185     190

Gly Ser Glu Gln Ala Val Ser Pro Glu Phe Thr Cys Ser Asn Gly Arg
195     200     205

Leu Ser Asn Ala Leu Asp Phe Pro Phe Asn Tyr Val Asp Ala Ile Ala
210     215     220

Asp Asn Glu Ile Val Ser Arg Leu Leu Gly Gly Asn Gln Met Trp Ser
225     230     235     240

Thr Thr Leu Asp Pro Leu Val Val Arg Gln Gly Thr Phe
245     250

<210> 41
<211> 1479
<212> DNA
<213> Arabidopsis thaliana

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## MBI16 Sequence Listing.ST25

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 <222> (192)..(962)  
 <223> G765

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 cgatctcttc aaaaagttaa tgttttcttg aaggattttt cttgttcttg atcaagcata 180  
 catatatata g atg gtg gaa gaa ggc ggc gta gtt gtg aat caa gga gga 230  
                   Met Val Glu Glu Gly Gly Val Val Val Asn Gln Gly Gly  
                   1                  5                  10  
 gat cag gag gtg gtg gat ttg cct ccg ggg ttt cgg ttt cac cct act 278  
 Asp Gln Glu Val Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr  
                   15                  20                  25  
 gat gaa gag ata ata act cac tac ctc aaa gag aag gtc ttc aac atc 326  
 Asp Glu Glu Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile  
                   30                  35                  40                  45  
 cga ttt acc gcg gca gcg att ggt caa gcc gac ctc aac aag aac gag 374  
 Arg Phe Thr Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu  
                   50                  55                  60  
 cca tgg gat cta cca aag att gca aag atg ggg gag aag gag ttt tac 422  
 Pro Trp Asp Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr  
                   65                  70                  75  
 ttt ttc tgc cag agg gat cgg aag tat ccg acc ggg atg agg acg aac 470  
 Phe Phe Cys Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn  
                   80                  85                  90  
 cgt gcg acc gtg tct ggt tat tgg aag gcg acc ggg aag gac aag gag 518  
 Arg Ala Thr Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu  
                   95                  100                  105  
 atc ttt aga ggc aaa ggt tgt ctt gtt ggg atg aag aaa aca ctt gtt 566  
 Ile Phe Arg Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val  
                   110                  115                  120                  125  
 ttc tat aca gga aga gct cct aaa ggt gaa aag acc aat tgg gtt atg 614  
 Phe Tyr Thr Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met  
                   130                  135  
 cat gaa tat cgt ctt gat gga aaa tat tct tat cat aac ctc ccc aaa 662  
 His Glu Tyr Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys  
                   145                  150                  155  
 acc gca agg gat gaa tgg gtg gtg tgt agg gtt ttt cac aag aac gct 710  
 Thr Ala Arg Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala  
                   160                  165                  170  
 cct agt act aca atc act act aca aaa caa ctc tca agg att gat tct 758  
 Pro Ser Thr Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser  
                   175                  180                  185  
 ctt gat aac att gat cat ctc tta gac ttc tca tct ctc cct cct ctc 806  
 Leu Asp Asn Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu  
                   190                  195                  200                  205  
 ata gat ccg ggt ttc ttg ggt caa ccc gcc caa gct tct ccg gtg ccc 854  
 Ile Asp Pro Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro  
                   210                  215                  220  
 gtc aac aac acg atc tca aac ctg tct cca cca tcc tac aac cgc acc 902  
 Val Asn Asn Thr Ile Ser Asn Leu Ser Pro Pro Ser Tyr Asn Arg Thr  
                   225                  230                  235  
 agt cga caa cac tta cct tcc tac cca agc tct caa ttt ccc tta cca 950

## MBI16 Sequence Listing.ST25

Ser Arg Gln His Leu Pro Ser Tyr Pro Ser Ser Gln Phe Pro Leu Pro  
 240 245 250

ctc ggt ccc taa ttccggatct gatttcggct acggggcagg ttcaggcaat 1002  
 Leu Gly Pro 255

aataacaaag gtatgatcaa gttggagcat tctcttgtga gtgtgtctca agaaaccggt 1062

ttgagttccg atgtgaacac aaccgcaacg ccagagatat cttcttatcc aatgatgatg 1122

aatccggcaa tgatggatgg tagcaagtca gcgtgtgatg gtcttgatga cttgatcttc 1182

tgggaagatt tatatactag cttaaatttg gaaaaggtta tttgttaatt gtgattgaag 1242

agtggcatat tgattactcg tctagtgttt ttaatcgtgt aattagttcg tatataatat 1302

acatgtacat aagatcatta gggtttattag gcattggact ttagttcggg gattgcttac 1362

ctagttttta gcttgagaaa aaaggctgtc attgggggta tgtttctttg tgattaactt 1422

gtacatatat acatttaaata taaacgtatg gtttaaatcg tttaaaaaaa aaaaaaa 1479

<210> 42

<211> 256

<212> PRT

<213> Arabidopsis thaliana

<400> 42

Met Val Glu Glu Gly Gly Val Val Val Asn Gln Gly Gly Asp Gln Glu  
 1 5 10 15

Val Val Asp Leu Pro Pro Gly Phe Arg Phe His Pro Thr Asp Glu Glu  
 20 25 30

Ile Ile Thr His Tyr Leu Lys Glu Lys Val Phe Asn Ile Arg Phe Thr  
 35 40 45

Ala Ala Ala Ile Gly Gln Ala Asp Leu Asn Lys Asn Glu Pro Trp Asp  
 50 55 60

Leu Pro Lys Ile Ala Lys Met Gly Glu Lys Glu Phe Tyr Phe Phe Cys  
 65 70 75 80

Gln Arg Asp Arg Lys Tyr Pro Thr Gly Met Arg Thr Asn Arg Ala Thr  
 85 90 95

Val Ser Gly Tyr Trp Lys Ala Thr Gly Lys Asp Lys Glu Ile Phe Arg  
 100 105 110

Gly Lys Gly Cys Leu Val Gly Met Lys Lys Thr Leu Val Phe Tyr Thr  
 115 120 125

Gly Arg Ala Pro Lys Gly Glu Lys Thr Asn Trp Val Met His Glu Tyr  
 130 135 140

Arg Leu Asp Gly Lys Tyr Ser Tyr His Asn Leu Pro Lys Thr Ala Arg  
 145 150 155 160

Asp Glu Trp Val Val Cys Arg Val Phe His Lys Asn Ala Pro Ser Thr  
 165 170 175

## MBI16 Sequence Listing.ST25

Thr Ile Thr Thr Thr Lys Gln Leu Ser Arg Ile Asp Ser Leu Asp Asn  
180 185 190

Ile Asp His Leu Leu Asp Phe Ser Ser Leu Pro Pro Leu Ile Asp Pro  
195 200 205

Gly Phe Leu Gly Gln Pro Ala Gln Ala Ser Pro Val Pro Val Asn Asn  
210 215 220

Thr Ile Ser Asn Leu Ser Pro Pro Ser Tyr Asn Arg Thr Ser Arg Gln  
225 230 235 240

His Leu Pro Ser Tyr Pro Ser Ser Gln Phe Pro Leu Pro Leu Gly Pro  
245 250 255

<210> 43  
<211> 825  
<212> DNA  
<213> Arabidopsis thaliana

<220>  
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<222> (1)..(825)  
<223> G197

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Met Gly Arg Ser Pro Cys Cys Glu Lys Asp His Thr Asn Lys Gly Ala  
1 5 10 15  
tgg act aag gaa gaa gac gat aag ctc atc tct tac atc aaa gct cac 96  
Trp Thr Lys Glu Glu Asp Asp Lys Leu Ile Ser Tyr Ile Lys Ala His  
20 25 30  
ggg gaa ggt tgt tgg cgt tct ctt cct aga tcc gcc ggt ctt caa cgt 144  
Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg  
35 40 45  
tgc gga aaa agc tgt cgt ctc cga tgg att aac tat ctc cga cct gat 192  
Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp  
50 55 60  
ctc aag agg ggt aac ttc acc ctc gaa gaa gat gat ctc atc atc aaa 240  
Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys  
65 70 75 80  
cta cat agc ctt ctc ggt aac aag tgg tct ctt att gcg acg aga tta 288  
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu  
85 90 95  
cca gga aga aca gat aac gag att aag aat tac tgg aac aca cat gtt 336  
Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val  
100 105 110  
aag agg aag cta tta aga aaa ggg att gat ccg gcg act cat cga cct 384  
Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro  
115 120 125  
atc aac gag acc aaa act tct caa gat tcg tct gat tct agt aaa aca 432  
Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr  
130 135 140  
gag gac cct ctt gtc aag att ctc tct ttt ggt cct cag ctg gag aaa 480  
Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys  
145 150 155 160  
ata gca aat ttc ggg gac gag aga att caa aag aga gtt gag tac tca 528  
Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser  
165 170 175



## MBI16 Sequence Listing.ST25

gtt gtt gaa gaa aga tgt ctg gac ttg aat ctt gag ctt agg atc agt 576  
Val Val Glu Glu Arg Cys Leu Asp Leu Asn Leu Glu Leu Arg Ile Ser  
180 185 190

cca cca tgg caa gac aag ctc cat gat gag agg aac cta agg ttt ggg 624  
Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly  
195 200 205

aga gtg aag tat agg tgc agt gcg tgc cgt ttt gga ttc ggg aac ggc 672  
Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly  
210 215 220

aag gag tgt agc tgt aat aat gtg aaa tgt caa aca gag gac agt agt 720  
Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser  
225 230 235 240

agc agc agt tat tct tca acc gac att agt agt agc att ggt tat gac 768  
Ser Ser Ser Tyr Ser Ser Thr Asp Ile Ser Ser Ser Ile Gly Tyr Asp  
245 250 255

ttc ttg ggt cta aac aac act agg gtt ttg gat ttt agc act ttg gaa 816  
Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu  
260 265 270

atg aaa tga 825  
Met Lys

<210> 44  
<211> 274  
<212> PRT  
<213> Arabidopsis thaliana

<400> 44

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Trp Thr Lys Glu Glu Asp Asp Lys Leu Ile Ser Tyr Ile Lys Ala His  
20 25 30

Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gln Arg  
35 40 45

Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp  
50 55 60

Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu Ile Ile Lys  
65 70 75 80

Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Thr Arg Leu  
85 90 95

Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Val  
100 105 110

Lys Arg Lys Leu Leu Arg Lys Gly Ile Asp Pro Ala Thr His Arg Pro  
115 120 125

Ile Asn Glu Thr Lys Thr Ser Gln Asp Ser Ser Asp Ser Ser Lys Thr  
130 135 140

Glu Asp Pro Leu Val Lys Ile Leu Ser Phe Gly Pro Gln Leu Glu Lys  
145 150 155 160

## MBI16 Sequence Listing.ST25

Ile Ala Asn Phe Gly Asp Glu Arg Ile Gln Lys Arg Val Glu Tyr Ser  
 165 170 175

Val Val Glu Glu Arg Cys Leu Asp Leu Asn Leu Glu Leu Arg Ile Ser  
 180 185 190

Pro Pro Trp Gln Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly  
 195 200 205

Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gly  
 210 215 220

Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gln Thr Glu Asp Ser Ser  
 225 230 240

Ser Ser Ser Tyr Ser Ser Thr Asp Ile Ser Ser Ser Ile Gly Tyr Asp  
 245 250 255

Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu  
 260 265 270

Met Lys

<210> 45  
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 <212> DNA  
 <213> Arabidopsis thalina

<220>  
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 <222> (30)..(839)  
 <223> G255

<400> 45  
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 Lys Glu His Met Asn Lys Gly Ala Trp Thr Lys Glu Glu Asp Glu Arg  
 10 15 20

cta gtc tct tac atc aag tct cac ggt gaa ggt tgt tgg cga tct ctt 149  
 Leu Val Ser Tyr Ile Lys Ser His Gly Glu Gly Cys Trp Arg Ser Leu  
 25 30 35 40

cct aga gcc gct ggt ctc ctt cgc tgc ggt aaa agc tgc cgt ctt cgg 197  
 Pro Arg Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser Cys Arg Leu Arg  
 45 50 55

tgg att aac tat ctc cga cct gat ctc aaa aga gga aac ttt aca cat 245  
 Trp Ile Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Asn Phe Thr His  
 60 65 70

gat gaa gat gaa ctt atc atc aag ctt cat agc ctc cta ggc aac aag 293  
 Asp Glu Asp Glu Leu Ile Ile Lys Leu His Ser Leu Leu Gly Asn Lys  
 75 80 85

tgg tct ttg att gcg gcg aga tta cct gga aga aca gat aac gag atc 341  
 Trp Ser Leu Ile Ala Ala Arg Leu Pro Gly Arg Thr Asp Asn Glu Ile  
 90 95 100

aag aac tac tgg aac aca cat ata aag agg aag ctt ttg agc aaa ggg 389

## MBI16 Sequence Listing.ST25

Lys Asn Tyr Trp Asn Thr His Ile Lys Arg Lys Leu Leu Ser Lys Gly  
 105 110 115 120  
 att gat cca gcc act cat aga ggg atc aac gag gca aaa att tct gat 437  
 Ile Asp Pro Ala Thr His Arg Gly Ile Asn Glu Ala Lys Ile Ser Asp  
 125 130 135  
 ttg aag aaa aca aag gac caa att gta aaa gat gtt tct ttt gtg aca 485  
 Leu Lys Lys Thr Lys Asp Gln Ile Val Lys Asp Val Ser Phe Val Thr  
 140 145 150  
 aag ttt gag gaa aca gac aag tct ggg gac cag aag caa aat aag tat 533  
 Lys Phe Glu Glu Thr Asp Lys Ser Gly Asp Gln Lys Gln Asn Lys Tyr  
 155 160 165  
 att cga aat ggg tta gtt tgc aaa gaa gag aga gtt gtt gtt gaa gaa 581  
 Ile Arg Asn Gly Leu Val Cys Lys Glu Glu Arg Val Val Val Glu Glu  
 170 175 180  
 aaa ata ggc cca gat ttg aat ctt gag ctt agg atc agt cca cca tgg 629  
 Lys Ile Gly Pro Asp Leu Asn Leu Glu Leu Arg Ile Ser Pro Pro Trp  
 185 190 195 200  
 caa aac cag aga gaa ata tct act tgc act gcg tcc cgt ttt tac atg 677  
 Gln Asn Gln Arg Glu Ile Ser Thr Cys Thr Ala Ser Arg Phe Tyr Met  
 205 210 215  
 gaa aac gac atg gag tgt agt agt gaa act gtg aaa tgt caa aca gag 725  
 Glu Asn Asp Met Glu Cys Ser Ser Glu Thr Val Lys Cys Gln Thr Glu  
 220 225 230  
 aat agt agc agc att agc tat tct tct att gat att agt agt agt aac 773  
 Asn Ser Ser Ser Ile Ser Tyr Ser Ser Ile Asp Ile Ser Ser Ser Asn  
 235 240 245  
 gtt ggt tat gac ttc ttg ggt ttg aag aca aga att ttg gat ttt cga 821  
 Val Gly Tyr Asp Phe Leu Gly Leu Lys Thr Arg Ile Leu Asp Phe Arg  
 250 255 260  
 agc ttg gaa atg aaa taa atgaatagta ttagattctt aattttagg 869  
 Ser Leu Glu Met Lys  
 265  
 tctgataatg aatgtagat tcgcggccct ctagacaggc ctcgtaccg 918

<210> 46  
 <211> 269  
 <212> PRT  
 <213> Arabidopsis thalina

<400> 46

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 Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ser Tyr Ile Lys Ser His  
 20 25 30  
 Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ala Ala Gly Leu Leu Arg  
 35 40 45  
 Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp  
 50 55 60  
 Leu Lys Arg Gly Asn Phe Thr His Asp Glu Asp Glu Leu Ile Ile Lys  
 65 70 75 80  
 Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu Ile Ala Ala Arg Leu  
 85 90 95

## MBI16 Sequence Listing.ST25

Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Thr His Ile  
 100 105 110

Lys Arg Lys Leu Leu Ser Lys Gly Ile Asp Pro Ala Thr His Arg Gly  
 115 120 125

Ile Asn Glu Ala Lys Ile Ser Asp Leu Lys Lys Thr Lys Asp Gln Ile  
 130 135 140

Val Lys Asp Val Ser Phe Val Thr Lys Phe Glu Glu Thr Asp Lys Ser  
 145 150 155 160

Gly Asp Gln Lys Gln Asn Lys Tyr Ile Arg Asn Gly Leu Val Cys Lys  
 165 170 175

Glu Glu Arg Val Val Val Glu Glu Lys Ile Gly Pro Asp Leu Asn Leu  
 180 185 190

Glu Leu Arg Ile Ser Pro Pro Trp Gln Asn Gln Arg Glu Ile Ser Thr  
 195 200 205

Cys Thr Ala Ser Arg Phe Tyr Met Glu Asn Asp Met Glu Cys Ser Ser  
 210 215 220

Glu Thr Val Lys Cys Gln Thr Glu Asn Ser Ser Ser Ile Ser Tyr Ser  
 225 230 235 240

Ser Ile Asp Ile Ser Ser Ser Asn Val Gly Tyr Asp Phe Leu Gly Leu  
 245 250 255

Lys Thr Arg Ile Leu Asp Phe Arg Ser Leu Glu Met Lys  
 260 265

<210> 47  
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 <223> G1113

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 Pro Thr Asp Phe Lys Glu Leu Gln Ile Pro Gly Tyr Val Leu Lys Thr  
 5 10 15

ctt tac gtc atc ggt ttc ttt aga gac atg gtc gat gct ctt tgt cct 152  
 Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp Ala Leu Cys Pro  
 20 25 30 35

tac atc ggt cta cca agt ttt ctt gac cac aac gag acc tct cga tcc 200  
 Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu Thr Ser Arg Ser  
 40 45 50

gac ccg acc cga ctc gct ctc tcc acg tca gca act ctt gcc aac gag 248

## MBI16 Sequence Listing.ST25

Asp Pro Thr Arg Leu Ala Leu Ser Thr Ser Ala Thr Leu Ala Asn Glu  
 55 60 65  
 tta atc ccg gtg gtt cgt ttc tcc gat ctt tta acc gat ccg gaa gat 296  
 Leu Ile Pro Val Val Arg Phe Ser Asp Leu Leu Thr Asp Pro Glu Asp  
 70 75 80  
 tgc tgc acg gtt tgc tta tcc gat ttt gta tcc gac gat aag att aga 344  
 Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp Lys Ile Arg  
 85 90 95  
 cag ctg ccg aag tgt gga cac gtg ttt cat cat cgt tgt tta gac cgt 392  
 Gln Leu Pro Lys Cys Gly His Val Phe His His Arg Cys Leu Asp Arg  
 100 105 110 115  
 tgg atc gtt gac tgt aat aag ata acg tgc ccg att tgt cgg aac cgg 440  
 Trp Ile Val Asp Cys Asn Lys Ile Thr Cys Pro Ile Cys Arg Asn Arg  
 120 125 130  
 ttc tta ccg gag gaa aag tcc acg ccg ttt gat tgg ggt act tca gat 488  
 Phe Leu Pro Glu Glu Lys Ser Thr Pro Phe Asp Trp Gly Thr Ser Asp  
 135 140 145  
 tgg ttt aga gat gaa gtg gag agt acc aac taa taatgatggg tttactttta 541  
 Trp Phe Arg Asp Glu Val Glu Ser Thr Asn  
 150 155  
 ctttttactt ttttcacggg aatatttttc tactgtataa ttctttcttc caaactactg 601  
 tataattcaa gtataagatt atgtaattgt gtatattagc atcaatcatc tttctttgt 660  
  
 <210> 48  
 <211> 157  
 <212> PRT  
 <213> Arabidopsis thaliana  
  
 <400> 48  
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 Leu Lys Thr Leu Tyr Val Ile Gly Phe Phe Arg Asp Met Val Asp Ala  
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 Leu Cys Pro Tyr Ile Gly Leu Pro Ser Phe Leu Asp His Asn Glu Thr  
 35 40 45  
 Ser Arg Ser Asp Pro Thr Arg Leu Ala Leu Ser Thr Ser Ala Thr Leu  
 50 55 60  
 Ala Asn Glu Leu Ile Pro Val Val Arg Phe Ser Asp Leu Leu Thr Asp  
 65 70 75 80  
 Pro Glu Asp Cys Cys Thr Val Cys Leu Ser Asp Phe Val Ser Asp Asp  
 85 90 95  
 Lys Ile Arg Gln Leu Pro Lys Cys Gly His Val Phe His His Arg Cys  
 100 105 110  
 Leu Asp Arg Trp Ile Val Asp Cys Asn Lys Ile Thr Cys Pro Ile Cys  
 115 120 125  
 Arg Asn Arg Phe Leu Pro Glu Glu Lys Ser Thr Pro Phe Asp Trp Gly  
 130 135 140

Thr Ser Asp Trp Phe Arg Asp Glu Val Glu Ser Thr Asn  
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## MBI16 Sequence Listing.ST25

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           220                          225                          230

agt cca ccg acc aca ctt ttg atg tgt cca tcg tgt gaa cgt gtg gcc 894  
 Ser Pro Pro Thr Thr Leu Leu Met Cys Pro Ser Cys Glu Arg Val Ala  
           235                          240                          245

gga cca tcc tca tct aac cac aac cag cga tct gtc tca ttg agt cca 942  
 Gly Pro Ser Ser Ser Asn His Asn Gln Arg Ser Val Ser Leu Ser Pro  
           250                          255                          260                          265

tgg ctc caa atg gcc cat ggg tca acc ttt gat gtg atg cgt cct agg 990  
 Trp Leu Gln Met Ala His Gly Ser Thr Phe Asp Val Met Arg Pro Arg  
                           270                          275                          280

tct taa ctttaatgct gcttctatgg gttgtgtgtg ggtcattgta ctttttagat 1046  
 Ser

tattgactct cagctaattgt atccttaaaa gcctttttct actttttaaatt ttactttaat 1106

ctaatttaaatt tagttgtcca tgtcttcttg ataacaaaaa aatttataat tataaaaaaa 1166

aaaaacagga taaaaaaaaa aaaaaaaaaa aaaaaa 1201

<210> 50  
 <211> 282  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 50

Met Met Met Gly Lys Glu Asp Leu Gly Leu Ser Leu Ser Leu Gly Phe  
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Ala Gln Asn His Pro Leu Gln Leu Asn Leu Lys Pro Thr Ser Ser Pro  
           20                          25                          30

Met Ser Asn Leu Gln Met Phe Pro Trp Asn Gln Thr Leu Val Ser Ser  
           35                          40                          45

Ser Asp Gln Gln Lys Gln Gln Phe Leu Arg Lys Ile Asp Val Asn Ser  
           50                          55                          60

Leu Pro Thr Thr Val Asp Leu Glu Glu Glu Thr Gly Val Ser Ser Pro  
           65                          70                          75                          80

Asn Ser Thr Ile Ser Ser Thr Val Ser Gly Lys Arg Arg Ser Thr Glu  
                           85                          90                          95

Arg Glu Gly Thr Ser Gly Gly Gly Cys Gly Asp Asp Leu Asp Ile Thr  
           100                          105                          110

Leu Asp Arg Ser Ser Ser Arg Gly Thr Ser Asp Glu Glu Glu Asp Tyr  
           115                          120                          125

Gly Gly Glu Thr Cys Arg Lys Lys Leu Arg Leu Ser Lys Asp Gln Ser  
           130                          135                          140

Ala Val Leu Glu Asp Thr Phe Lys Glu His Asn Thr Leu Asn Pro Lys  
           145                          150                          155                          160

## MBI16 Sequence Listing.ST25

Gln Lys Leu Ala Leu Ala Lys Lys Leu Gly Leu Thr Ala Arg Gln Val  
165 170 175

Glu Val Trp Phe Gln Asn Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr  
180 185 190

Glu Val Asp Cys Glu Tyr Leu Lys Arg Cys Val Glu Lys Leu Thr Glu  
195 200 205

Glu Asn Arg Arg Leu Glu Lys Glu Ala Ala Glu Leu Arg Ala Leu Lys  
210 215 220

Leu Ser Pro Arg Leu Tyr Gly Gln Met Ser Pro Pro Thr Thr Leu Leu  
225 230 235 240

Met Cys Pro Ser Cys Glu Arg Val Ala Gly Pro Ser Ser Ser Asn His  
245 250 255

Asn Gln Arg Ser Val Ser Leu Ser Pro Trp Leu Gln Met Ala His Gly  
260 265 270

Ser Thr Phe Asp Val Met Arg Pro Arg Ser  
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<211> 937

<212> DNA

<213> Arabidopsis thaliana

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<222> (120)..(797)

<223> G395

<400> 51

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atg ccc tta gga gca gct acg gtt gtg gag gag gaa gag gag gag gag 167  
Met Pro Leu Gly Ala Ala Thr Val Val Glu Glu Glu Glu Glu Glu  
1 5 10 15

gaa gcg gtg cct agt atg tca gta tcg ccg ccg gat agt gta acg tcg 215  
Glu Ala Val Pro Ser Met Ser Val Ser Pro Pro Asp Ser Val Thr Ser  
20 25 30

tcg ttt caa ttg gac ttt ggg att aaa agt tat ggt tat gag aga aga 263  
Ser Phe Gln Leu Asp Phe Gly Ile Lys Ser Tyr Gly Tyr Glu Arg Arg  
35 40 45

agc aat aag aga gat att gat gat gaa gtg gag aga tca gcc tca aga 311  
Ser Asn Lys Arg Asp Ile Asp Asp Glu Val Glu Arg Ser Ala Ser Arg  
50 55 60

gcc agc aac gaa gac aac gat gac gag aat gga tcc act agg aag aaa 359  
Ala Ser Asn Glu Asp Asn Asp Asp Glu Asn Gly Ser Thr Arg Lys Lys  
65 70 75 80

ctt aga ctc tcc aaa gac caa tct gct ttt ctt gaa gac agc ttc aaa 407  
Leu Arg Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Asp Ser Phe Lys  
85 90 95

gaa cac agt acc ctt aat cct aaa cag aag att gca ttg gcg aag cag 455  
Glu His Ser Thr Leu Asn Pro Lys Gln Lys Ile Ala Leu Ala Lys Gln  
100 105 110



## MBI16 Sequence Listing.ST25

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ttg aat ctt cgt cct cgt cag gtt gaa gtc tgg ttt caa aac aga cga      503
Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg
      115                      120                      125

gcc agg aca aag ctg aag caa acg gaa gtg gac tgt gaa tac cta aag      551
Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys
      130                      135                      140

aga tgc tgt gag tca cta acc gaa gaa aac cgg agg ctt caa aaa gag      599
Arg Cys Cys Glu Ser Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu
      145                      150                      155                      160

gtt aaa gaa ttg aga acc ttg aag act tcc aca ccc ttt tac atg caa      647
Val Lys Glu Leu Arg Thr Leu Lys Thr Ser Thr Pro Phe Tyr Met Gln
      165                      170                      175

ctt ccg gcc act act ctc act atg tgc cct tct tgt gaa cgt gtt gcc      695
Leu Pro Ala Thr Thr Leu Thr Met Cys Pro Ser Cys Glu Arg Val Ala
      180                      185                      190

act tca gca gca cag ccc tcc acg tca gct gcc cac aac ctc tgt ttg      743
Thr Ser Ala Ala Gln Pro Ser Thr Ser Ala Ala His Asn Leu Cys Leu
      195                      200                      205

tcc acg tca tca ttg att ccg gtt aag cct cgg ccg gcc aaa caa gtt      791
Ser Thr Ser Ser Leu Ile Pro Val Lys Pro Arg Pro Ala Lys Gln Val
      210                      215                      220

tca tga aagcacctgc gaaatacagt ttgagcaaac gggcggccgc tctagacagg      847
Ser
225

cctcgtaccg gatcctctag ctagagcttt cgttcgtatc atcgggtttcg acaacgttcg      907

tcaagttcaa tgacatcagt ttgattgcgc      937

<210> 52
<211> 225
<212> PRT
<213> Arabidopsis thaliana

<400> 52

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Glu Ala Val Pro Ser Met Ser Val Ser Pro Pro Asp Ser Val Thr Ser
20      25      30

Ser Phe Gln Leu Asp Phe Gly Ile Lys Ser Tyr Gly Tyr Glu Arg Arg
35      40      45

Ser Asn Lys Arg Asp Ile Asp Asp Glu Val Glu Arg Ser Ala Ser Arg
50      55      60

Ala Ser Asn Glu Asp Asn Asp Asp Glu Asn Gly Ser Thr Arg Lys Lys
65      70      75      80

Leu Arg Leu Ser Lys Asp Gln Ser Ala Phe Leu Glu Asp Ser Phe Lys
85      90      95

Glu His Ser Thr Leu Asn Pro Lys Gln Lys Ile Ala Leu Ala Lys Gln
100     105     110

Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn Arg Arg
115     120     125

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## MBI16 Sequence Listing.ST25

Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Tyr Leu Lys  
130 135 140

Arg Cys Cys Glu Ser Leu Thr Glu Glu Asn Arg Arg Leu Gln Lys Glu  
145 150 155 160

Val Lys Glu Leu Arg Thr Leu Lys Thr Ser Thr Pro Phe Tyr Met Gln  
165 170 175

Leu Pro Ala Thr Thr Leu Thr Met Cys Pro Ser Cys Glu Arg Val Ala  
180 185 190

Thr Ser Ala Ala Gln Pro Ser Thr Ser Ala Ala His Asn Leu Cys Leu  
195 200 205

Ser Thr Ser Ser Leu Ile Pro Val Lys Pro Arg Pro Ala Lys Gln Val  
210 215 220

Ser  
225

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<223> G393

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Cys Asn Thr Gly Leu Val Leu Gly Leu Gly Pro Ser Pro Ile Ser Asn  
10 15 20

aat tac aat agt acc atc aga caa tcc tcc gtt tac aag ctc gag ccg 150  
Asn Tyr Asn Ser Thr Ile Arg Gln Ser Ser Val Tyr Lys Leu Glu Pro  
25 30 35

tcg ttg act cta tgc ctc tcg ggc gat ccc tcg gtt acc gtg gtg acc 198  
Ser Leu Thr Leu Cys Leu Ser Gly Asp Pro Ser Val Thr Val Val Thr  
40 45 50

gga gct gac cag cta tgc cgt cag acg tca tct cac agc gga gtc tct 246  
Gly Ala Asp Gln Leu Cys Arg Gln Thr Ser Ser His Ser Gly Val Ser  
55 60 65 70

tct ttc tca agc ggg agg gtg gtg aaa aga gag aga gac ggt ggc gaa 294  
Ser Phe Ser Ser Arg Val Val Lys Arg Glu Arg Asp Gly Gly Glu  
75 80 85

gag tcg ccg gag gag gaa gag atg acg gag aga gtt ata agt gat tac 342  
Glu Ser Pro Glu Glu Glu Glu Met Thr Glu Arg Val Ile Ser Asp Tyr  
90 95 100

cat gaa gat gaa gaa ggt att agt gct aga aaa aaa ctt agg ctt acg 390  
His Glu Asp Glu Glu Gly Ile Ser Ala Arg Lys Lys Leu Arg Leu Thr  
105 110 115

aaa caa caa tct gct ctt ctt gag gaa agc ttc aag gat cat agc acc 438

MBI16 Sequence Listing.ST25

Lys	Gln	Gln	Ser	Ala	Leu	Leu	Glu	Glu	Ser	Phe	Lys	Asp	His	Ser	Thr	
120						125					130					
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Leu	Asn	Pro	Lys	Gln	Lys	Gln	Val	Leu	Ala	Arg	Gln	Leu	Asn	Leu	Arg	
135					140					145					150	
cct	aga	caa	ggt	gaa	gta	tgg	ttt	caa	aat	aga	aga	gcc	agg	aca	aag	534
Pro	Arg	Gln	Val	Glu	Val	Trp	Phe	Gln	Asn	Arg	Arg	Ala	Arg	Thr	Lys	
				155					160					165		
ctg	aag	caa	aca	gaa	gta	gat	tgt	gag	ttt	ttg	aag	aag	tgt	tgt	gaa	582
Leu	Lys	Gln	Thr	Glu	Val	Asp	Cys	Glu	Phe	Leu	Lys	Lys	Cys	Cys	Glu	
			170					175					180			
aca	tta	gca	gat	gag	aac	ata	aga	ctt	cag	aaa	gag	att	caa	gaa	ctc	630
Thr	Leu	Ala	Asp	Glu	Asn	Ile	Arg	Leu	Gln	Lys	Glu	Ile	Gln	Glu	Leu	
		185					190					195				
aaa	acc	cta	aaa	ttg	act	cag	ccc	ttt	tac	atg	cac	atg	cct	gca	tcg	678
Lys	Thr	Leu	Lys	Leu	Thr	Gln	Pro	Phe	Tyr	Met	His	Met	Pro	Ala	Ser	
	200					205					210					
act	cta	acg	aag	tgt	cct	tct	tgt	gag	aga	atc	ggc	ggc	ggc	ggc	ggg	726
Thr	Leu	Thr	Lys	Cys	Pro	Ser	Cys	Glu	Arg	Ile	Gly	Gly	Gly	Gly	Gly	
215					220					225					230	
ggt	aat	gga	gga	gga	ggt	ggc	ggc	agc	ggg	gct	acc	gcg	gtg	att	gta	774
Gly	Asn	Gly	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Ala	Thr	Ala	Val	Ile	Val	
				235					240					245		
gat	gga	agt	acg	gcc	aaa	gga	gct	ttc	tct	atc	tcc	tca	aag	cct	cac	822
Asp	Gly	Ser	Thr	Ala	Lys	Gly	Ala	Phe	Ser	Ile	Ser	Ser	Lys	Pro	His	
			250					255					260			
ttc	ttc	aac	cct	ttt	act	aac	cca	tct	gca	gct	tgt	tga	atag	taatt		871
Phe	Phe	Asn	Pro	Phe	Thr	Asn	Pro	Ser	Ala	Ala	Cys					
		265					270									
cg	ttta	attt	tatt	act	ttaa	aatat	taatt	ttct	tttt	tttt	gggt	ggg	catt	ttt		927

<210> 54  
 <211> 274  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 54

Met	Gly	Phe	Asp	Asp	Thr	Cys	Asn	Thr	Gly	Leu	Val	Leu	Gly	Leu	Gly	
1			5						10					15		
Pro	Ser	Pro	Ile	Ser	Asn	Asn	Tyr	Asn	Ser	Thr	Ile	Arg	Gln	Ser	Ser	
			20					25					30			
Val	Tyr	Lys	Leu	Glu	Pro	Ser	Leu	Thr	Leu	Cys	Leu	Ser	Gly	Asp	Pro	
		35					40					45				
Ser	Val	Thr	Val	Val	Thr	Gly	Ala	Asp	Gln	Leu	Cys	Arg	Gln	Thr	Ser	
	50					55					60					
Ser	His	Ser	Gly	Val	Ser	Ser	Phe	Ser	Ser	Gly	Arg	Val	Val	Lys	Arg	
65					70					75				80		
Glu	Arg	Asp	Gly	Gly	Glu	Glu	Ser	Pro	Glu	Glu	Glu	Glu	Met	Thr	Glu	
			85						90					95		
Arg	Val	Ile	Ser	Asp	Tyr	His	Glu	Asp	Glu	Glu	Gly	Ile	Ser	Ala	Arg	
			100					105						110		

## MBI16 Sequence Listing.ST25

Lys Lys Leu Arg Leu Thr Lys Gln Gln Ser Ala Leu Leu Glu Glu Ser  
 115 120 125  
 Phe Lys Asp His Ser Thr Leu Asn Pro Lys Gln Lys Gln Val Leu Ala  
 130 135 140  
 Arg Gln Leu Asn Leu Arg Pro Arg Gln Val Glu Val Trp Phe Gln Asn  
 145 150 155 160  
 Arg Arg Ala Arg Thr Lys Leu Lys Gln Thr Glu Val Asp Cys Glu Phe  
 165 170 175  
 Leu Lys Lys Cys Cys Glu Thr Leu Ala Asp Glu Asn Ile Arg Leu Gln  
 180 185 190  
 Lys Glu Ile Gln Glu Leu Lys Thr Leu Lys Leu Thr Gln Pro Phe Tyr  
 195 200 205  
 Met His Met Pro Ala Ser Thr Leu Thr Lys Cys Pro Ser Cys Glu Arg  
 210 215 220  
 Ile Gly Gly Gly Gly Gly Gly Asn Gly Gly Gly Gly Gly Gly Ser Gly  
 225 230 235 240  
 Ala Thr Ala Val Ile Val Asp Gly Ser Thr Ala Lys Gly Ala Phe Ser  
 245 250 255  
 Ile Ser Ser Lys Pro His Phe Phe Asn Pro Phe Thr Asn Pro Ser Ala  
 260 265 270  
 Ala Cys

## INTERNATIONAL SEARCH REPORT

In application No.

PCT/US00/31458

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 5/04, 5/10, 15/00, 15/09, 15/63, 15/70, 15/74, 15/82, 15/87; C07H 21/02, 21/04; A01H 1/00, 9/00, 11/00  
 US CL : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
 U.S. : 435/320.1, 419, 468; 536/23.1; 800/ 278, 295

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X ---	Database GenEmbl on STIC, USPTO, (Arlington, VA, USA), GenBank Accession AC002388, LIN et al. 'Sequence analysis of chromosome 2 of the plant Arabidopsis thaliana,' abstract, Nature, 1999, Vol. 402, 761-768.	4-6 ----- 1-3, 7-13, 25-27
P,Y ---	Database EST on STIC, USPTO, (Arlington, VA, USA), GenBank Accession AV552445, ASAMIZU et al. 'A large scale analysis of cDNA in Arabidopsis thaliana: generation of 12,028 non-redundant expressed sequence tags from normalized and size-selected cDNA libraries,' abstract, DNA Research, 2000, Vol. 7, 175-180.	4-6 ----- 1-3, 7-13, 25-27
X --- Y	Database EST on STIC, USPTO, (Arlington, VA, USA), Genbank Accession AI997809, CHEN et al. unpublished, abstract, 08 September 1999.	4-6 ----- 1-3, 7-13, 25-27
X --- Y	Database EST on STIC, USPTO, (Arlington, VA, USA), GenBank Accession N97133, NEWMAN et al. 'Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones,' abstract, Plant Physiology, 1994, Vol. 106, 1241-1255.	4-6 ----- 1-3, 7-13, 25-27



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

13 February 2001 (13.02.2001)

Date of mailing of the international search report

07 MAR 2001

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

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# INTERNATIONAL SEARCH REPORT

I. application No.

PCT/US00/31458

## C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database EST on STIC, USPTO, (Arlington, VA, USA). GenBank Accession AA598183, NEWMAN et al. 'Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones,' abstract, Plant Physiology, 1994, Vol. 106, 1241-1255.	4-6 ----- 1-3, 7-13, 25-27
X --- Y	Database PIR_66 on STIC, USPTO, (Arlington, VA, USA), Accession T00409, ROUNSLEY et al. unpublished, abstract, 01 February 1999.	11 ----- 1-10, 12-13, 25-27
X --- Y	Database SPTREMBL_15 on STIC, USPTO, (Arlington, VA, USA), Accession 022167, ROUNSLEY et al. unpublished, abstract, 01 January 1998.	11 ----- 1-10, 12-13, 25-27
T,E	RIECHMANN et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science. 15 December 2000, Vol. 290, pages 2105-2110.	
P,A	SUNG et al. Developmentally regulated expression of two MADS-box genes, MdMADS3 and MdMADS4, in the morphogenesis of flower buds and fruits in apple. Planta. March 2000, Vol. 210, pages 519-528.	
P,Y	RIECHMANN et al. A genomic perspective on plant transcription factors. Current Opinion in Plant Biology. October 2000, Vol. 3, pages 423-434, especially pages 427-428.	1-13, 25-27
Y	US 5,892,009 A (THOMASHOW et al.) 06 April 1999, column 14, lines 1-46.	1-3, 7-10, 12-13, 25-27
A	RATCLIFFE et al. Separation of shoot and floral identity in Arabidopsis. Development. March 1999, Vol. 126, pages 1109-1120.	
A	SUNG et al. Characterization of MdMADS2, a member of the SQUAMOSA subfamily of genes, in apple. Plant Physiology. August 1999, Vol. 120, pages 969-978.	
A	RIECHMANN et al. The AP2/EREBP family of plant transcription factors. Biol. Chem. June 1998, Vol. 379, pages 633-646.	
A	RIECHMANN et al. Determination of floral organ identity by Arabidopsis MADS domain homeotic proteins AP1, AP3, PI, and AG is independent of their DNA-binding specificity. Molecular Biology of the Cell. July 1997, Vol., pages 1243-1259.	
A	HEARD et al. Evolutionary diversity of symbiotically induced nodule MADS box genes: characterization of nmhC5, a member of a novel subfamily. Molecular Plant-Microbe Interactions. July 1997, Vol. 10, No. 5, pages 665-676.	
A	RIECHMANN et al. MADS domain proteins in plant development. Biol. Chem. October 1997, Vol. 378, pages 1079-1101.	
A	RIECHMANN et al. DNA-binding properties of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA and AGAMOUS. Nucleic Acids Research. August 1996, Vol. 24, No. 16, pages 3134-3141.	
A	RIECHMANN et al. Dimerization specificity of Arabidopsis MADS domain homeotic proteins APETALA1, APETALA3, PISTILLATA, and AGAMOUS. Proc. Natl. Acad. Sci. USA. May 1996, Vol. 93, pages 4793-4798.	
A	HEARD et al. Symbiotic induction of a MADS-box gene during development of alfalfa root nodules. Proc. Natl. Acad. Sci. USA. June 1995, Vol. 92, pages 5273-5277.	

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31458

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13, 25-27 SEQ ID NOS: 1 and 2

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/31458

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING** This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I-XXVII, claim(s) 1-13 and 25-27, drawn to transgenic plants with modified environmental stress tolerance, polynucleotides and vectors for producing said transgenic plants, and methods of making said transgenic plants. Applicant must elect one pair of sequences (one nucleotide sequence and its corresponding amino acid translation) per Group to be examined, *i.e.* SEQ ID NOS: 1 and 2 in Group I, SEQ ID NOS: 3 and 4 in Group II, SEQ ID NOS: 5 and 6 in Group III, etc.

Group XXVIII, claim(s) 15-17, drawn to a method of identifying a factor that is modulated by or interacts with a polypeptide.

Group XXIX, claim(s) 18, drawn to a method of identifying a molecule that modulates activity or expression of a polynucleotide or polypeptide of interest.

Group XXX, claim(s) 19 and 20, drawn to an integrated system, computer, or computer readable medium.

Group XXXI, claim(s) 21-23, drawn to a method of identifying a polynucleotide sequence.

The inventions listed as Groups I-XXXI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I-XXVII are drawn to transgenic plants and methods of producing said plants with nucleic acid sequences. The methods of Groups I-XXVII differ from each other in that they are directed to plant transformation methods and transgenic plants with structurally and functionally distinct nucleic acid sequences which encode structurally and functionally different amino acid sequences. In addition, Groups XXVIII, XXIX, and XXXI are different methods from any of Groups I-XXVII in that they have different method steps and different end products, and Group XXX requires a computer system. Thus, there is no single special technical feature which links the inventions of Groups I-XXXI under PCT Rule 13.2.

**Continuation of B. FIELDS SEARCHED Item 3:** STN (agricola, biosis, biotechno, biotechds, biotechabs, caba, caplus, embase, medline, uspatfull, wpids, pctfull, europatfull, japio) SEARCH TERMS: inventor names, plant transcription factor, stress tolerance; STIC sequence search for SEQ ID NOS: 1 and 2